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# NEW ORGANIC MATERIALS ALTERNATIVE TO PEAT FOR A SUSTAINABLE NURSERY AND HORTICULTURE PRODUCTION

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DATA CONSEGNA TESI 31 gennaio 2012

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# Riassunto

La torba, grazie alle sue favorevoli caratteristiche chimico-fisiche, è il materiale più utilizzato nella preparazione delle miscele di substrati ma la crescente sensibilità verso la salvaguardia ambientale e la sostenibilità delle produzioni agricole hanno sollevato alcune problematiche di ordine ambientale ed economico. Per questi motivi si è osservato un crescente interesse nell'individuare materiali che possano anche parzialmente sostituire la torba garantendo però gli standard qualitativi richiesti per i substrati. Alcune biomasse di scarto dell'attività agro-industriale potrebbero essere impiegate allo scopo, come ammendanti o fertilizzanti nei substrati di coltivazione, diventando così una potenziale nuova risorsa. Tra queste la lolla di riso è un materiale facilmente disponibile in quantità consistenti. Questo sottoprodotto aumenta la porosità dei substrati ed è stato dimostrato essere un valido sostituto della perlite. I digestati anaerobici invece possono essere utilizzati in agricoltura come fertilizzanti o ammendanti perché generalmente presentano un elevato contenuto di elementi nutritivi e di sostanza organica.

L'obbiettivo di questo studio è stato di valutare l'effetto dell'uso della lolla di riso e di digestati anaerobici nella costituzione di substrati in parziale o totale sostituzione della torba. Lo studio ha riguardato la caratterizzazione fisica, chimica ed agronomica di substrati per la produzione di semenzali, per la radicazione di talee, per la coltivazione di ornamentali in vaso e per la radicazione e coltivazione di ornamentali in vaso di piccole dimensioni.

Partendo da substrati torbosi, per ciascuna prova si sono preparati 4 substrati in cui questi sono stati gradualmente sostituita con percentuali crescenti di lolla di riso macinata (0, 33, 67 e 100% rispettivamente). Altri 4 substrati sono stati preparati, a partire dai primi, aggiungendo digestati anaerobici di matrici vegetali (in seguito digestati anaerobici) come fertilizzante in ragione del 20% sul volume. Nella prova di coltivazione di ornamentali in vaso, agli 8 substrati prearati con le modalità descritte se ne sono aggiunti altri otto in cui la lolla di riso non era stata macinata.

Sotto il profilo fisico, la porosità dell'aria si è rivelata spesso eccessiva nei substrati con alte percentuali di lolla di riso a discapito dei volumi delle diverse frazioni che compongono la capacità di ritenzione idrica. I digestati anaerobici hanno marginalmente migliorato i parametri considerati e non sempre hanno presentato differenze significative rispetto i substrati non concimati.

Dosi crescenti di lolla di riso hanno, in generale, ridotto la capacità di scambio cationico, il contenuto percentuale in azoto, ma anche le concentrazioni di N-NO<sub>3</sub>, Ca e Mg nell'estratto acquoso. Viceversa si sono osservate crescenti concentrazioni di P e K. La lolla di riso dunque potrebbe aver creato degli squilibri tra gli elementi nutritivi mentre la fertilizzazione con i digestati anaerobici ha, in generale, aumentato la dotazione di tutti gli elementi nutritivi analizzati.

La prima serie di prove di coltivazione ha visto lo studio dell'effetto dei diversi substrati su semenzali di pomodoro 'Jack', *Salvia officinalis, Salvia splendens* 'Maestro' and *Begonia semperflorens* 'Super Olympia Red'. L'aumento della lolla di riso nei substrati in generale non ha influenzato la crescita del pomodoro (ad esclusione del negativo effetto dei substrati di sola lolla) e della *S. officinalis*, ma ha limitato quella delle due specie floricole in particolare quando superiore al 33%. L'aggiunta dei digestati anaerobici ha sostenuto la crescita di tutte le piante.

Una successiva prova di coltivazione su semenzali di pomodoro 'Jack' è stata allestita con lo scopo di valutare l'effetto sulle piante dell'adozione di 3 diversi regimi irrigui rispettivamente di 225 e 450 ml d<sup>-1</sup> per vassoio alveolato e 900 ml 2d<sup>-1</sup> per vassoio alveolato. L'irrigazione con 450 ml d<sup>-1</sup> ha permesso la maggior crescita delle piante mentre nessuna differenza significativa è stata osservata tra gli altri due trattamenti.

L'effetto dei substrati sulla radicazione è stato studiato utilizzando talee di *Rosa* ×*hybrida* 'La sevillana' e *Pelargonium hederifolium* 'Ville de Paris' fatte radicare utilizzando il mist e successivamente adattate all'ambiente di coltivazione. Sulle talee di rosa sono stati eseguiti due rilievi mentre soltanto uno su geranio. Sulla rosa già al primo rilievo si è osservato una riduzione del peso fresco e secco delle radici e della loro lunghezza aumentando il contenuto di lolla d riso nei substrati. Al termine dell'esperimento le differenze osservate per le radici si sono estese anche ai valori dello SPAD e ad alcuni pesi freschi e secchi dei diversi organi della parte aerea delle piante. Nuovamente l'aggiunta di digestati anaerobici ha permesso lo sviluppo di piante in media più grandi. Il geranio invece non ha evidenziato differenze significative relative all'aumento della lolla di riso, ma l'impiego dei digestati anaerobici ha in generale ridotto l'accrescimento delle talee di geranio.

Le prove relative allo studio della coltivazione di ornamentali in vaso hanno riguardato le stesse specie e varietà utilizzate nella prova di radicazione. Su geranio molti parametri vegetativi sono risultati significativamente influenzati dall'interazione "lolla di riso × digestati". Il peso fresco e secco delle radici è aumentato all'aumentare delle percentuale di lolla di riso nel substrato in particolare con il 33 e 67% in assenza di digestati anaerobici. I substrati con lolla di riso e senza digestati sono stati quelli con le caratteristiche fisico-chimiche più penalizzanti e questo può aver stimolato la produzione di un maggiore numero di radici. Tutti gli altri parametri vegetativi misurati sono diminuiti all'aumentare della percentuale di lolla di riso nel substrato. La macinazione della lolla ha sensibilmente ridotto l'effetto negativo probabilmente per le migliori proprietà fisiche. Le migliori piante di geranio sono state ottenute sui substrati testimoni, oppure sui substrati composti da torba e digestati. La coltivazione della rosa ha mostrato gli stessi trend decrescenti osservati in geranio con l'aumentare della percentuale di lolla di riso.

Un'ultima prova di coltivazione è stata fatta facendo radicare e coltivando direttamente sullo stesso vaso *Rosa* ×*hybrida* 'Tilt Meillandina'. In generale le piante, ad esclusione di quelle radicate sui substrati 67 e 100% di lolla e senza digestati, non sono risultate diverse da quelle coltivate sul substrato testimone. I buoni risultati ottenuti nella fase di coltivazione dell'utilizzo della lolla di riso in sostituzione alla torba sono stati fortemente penalizzati dai dati relativi alle percentuali di attecchimento e dal numero di vasi commerciabili ottenuti per ciascuna tesi. L'attecchimento delle talee infatti è risultato fortemente limitato sia dalla percentuale di lolla di riso che dalla presenza dei digestati anaerobici. Dalla percentuale di attecchimento poi è in parte dipesa quella delle piante commerciabili, che però è risultata indipendente dalla presenza di digestati e inferiore, rispetto, al testimone, solamente nei substrati privi di torba.

In conclusione l'aggiunta della lolla di riso ha modificato, generalmente peggiorando, la disponibilità di acqua ed aumentando anche notevolmente lo spazio per l'aria. Sotto il profilo chimico inoltre la lolla di riso ha spesso alterato il quadro nutrizionale. L'aggiunta dei digestati anaerobici ha confermato la loro potenzialità come fertilizzante da miscelare con i substrati aumentando la disponibilità di elementi nutritivi. Sotto l'aspetto pratico la sostituzione della torba con la lolla di riso sembra possibile per

alcune specie orticole ed ornamentali nella produzione di semenzali (pomodoro e *Salvia officinalis*) e di talee radicate (geranio) ma se ne sconsiglia l'impiego per le ornamentali più delicate con ciclo più lungo (*Salvia splendens*, *Begonia semperflorens* e rosa). La parziale sostituzione della torba con lolla di riso è possibile nelle coltivazioni a medio periodo in vaso soprattutto per la coltivazione della rosa. L'impiego di digestati anaerobici è risultato utile nella coltivazione di semenzali e di piante in vaso mentre è sconsigliabile nei substrati per la radicazione di geranio.

# Summary

Due to its favorable physico-chemical characteristics, peat is the most important material used in substrate mixes. Increasing attention to environmental issues and to sustainability of agricultural activities and increasing peat price have risen interest to alternative materials. Some agro-industrial biomasses can be used valorizing a possible waste. Among these, rice hulls are a by-product easily available in large amounts. It is used in substrates to improve air pore space and it was demonstrated that it is a proper perlite alternative. Anaerobic digestion residues may be used in agriculture as amendment or as fertilizer because generally have high organic matter and nutrient content.

The aim of this study vas to evaluate the effect rice hulls and anaerobic digestion residues of vegetal biomasses in substrates as partial or total substitute for peat. This study considered the physical, chemical and agronomical characterization of different substrates for transplant production, rooting cuttings production, potted ornamentals growth and for direct rooting a cultivation of potted ornamentals.

In each experiment, four substrates were prepared substituting peat with increasing rate of 2 mm ground rice hulls (GRH) (0, 33, 67 and 100% respectively). Four more substrates were prepared with the same proportions but adding 20% by volume of anaerobic digestion residues of vegetal biomasses (ADR). In the potted ornamentals experiment 8 more substrate were prepared using whole rice hulls.

Physical characterization pointed out that air pore space often resulted excessive with high GRH percentages reducing water holding capacity and its components. Anaerobic digestion residues slightly increased some of these latters parameters.

The increase of GRH generally reduced cation exchange capacity, total nitrogen content, nitrate nitrogen, Ca and Mg content and increased those of P and K. GRH may have caused nutrient disequilibrium but ADR addition increased nutrient level in all substrates.

Agronomic evaluation on 'Jack' tomato, *Salvia officinalis*, *Salvia splendens* 'Maestro' and *Begonia semperflorens* 'Super Olympia Red' transplants pointed out that increasing GRH content on substrates generally did not affected tomato and *S. officinalis* growth but reduced two flower species. ADR addition, instead, increased all plants growth. This responce is probably due to lower physical properties of substrates with increasing rate of GRH and to higher sensitiveness to water stress of two flower species.

A new cultivation experiment was carried out using 'Jack' tomato seedling under three different water managements (225 e 450 ml  $d^{-1}$  per tray and 900 ml  $2d^{-1}$  per tray). In this case watering with 450 ml  $d^{-1}$  allowes higher tomato seedling growth.

*Rosa* ×*hybrida* 'La sevillana' and *Pelargonium hederifolium* 'Ville de Paris' cuttings were rooted under mist. Two sampling were performed on rose while only one on geranium due to faster rooting. Since the first sampling, rose cutting rooting was negatively affected by increasing GRH content in substrates. In the final sampling the negative effect was stronger on SPAD values and fresh and dry matter of some organs of cutting. Once again ADR addition allowed better performances. On the contrary, geranium cuttings were not affected by GRH rate in the substrates but was negatively affected by ADR addition.

In the potted ornamental experiment *Pelargonium hederifolium* 'Ville de Paris' and *Rosa*  $\times$ *hybrida* 'La sevillana' were also used. Many geranium parameters were affected by "GRH  $\times$  ADR" interaction. Fresh and dry root weight increased along with the increase of GRH expecially in substrates with 33 and 67% without ADR. Substrates with GRH and without ADR had poorer physical properties and this may have stimulated higher root number. All the other vegetative parameters decreased increasing GRH content in the substrates. GRH grinding slightly reduced negative effect of increasing GRH content probably because of better physical properties. Geranium plants grown better in control substrates or in substrates with peat and ADR. Rose growth had the same decreasing trend observed in geranium with increasing GRH content but it resulted more tolerant to partial peat substitution.

Direct rooting and cultivation was done with *Rosa* ×*hybrida* 'Tilt Meillandina'. In this case slightly decreasing values of different parameters were observed increasing GRH content in substrates with higher rate of GRH not fertilized with ADR. With the exclusion of plants grown on substrate with 67 and 100% GRH ADRE-free, results obtained in the other substrates generally did not differ from those observed in the peat control substrate. However, the good result obtained during cultivation phase were remarkably reduced by rooting percentage and commercial pot percentage obtained in 100% GRH and in substraints containing ADR.

In conclusion GRH addition generally decreased water availability increasing air

pore space outlining the necessity to change watering strategy and modified chemical characteristic altering nutritional balance. ADR addition instead generally confirmed it good potential as fertilizer for substrates. Under practical aspect peat substitution with rice hulls seems possible to grow some vegetable transplant (tomato and *Salvia officinalis*) and rooting cuttings (geranium) but its use is not suggested for sensitive ornamentals with longer growing cycle (*Salvia splendens, Begonia semperflorens* and rose). Partial peat substitution with GRH is possible in potted cultivation especially for rose. ADR addition resulted useful for seedling transplants and potted ornamentals production but not for rooting substrate of geranium.

Chapter I

Introduction

## Introduction

#### Soil-less cultivation and substrates

Soil-less cultures have been successfully used for several decades with the aim of intensifying production and reducing costs (Maloupa et al., 1992). In this cultivation system, plants are grown without soil while water and fertilizers are applied with nutrient solutions. Soil-less cultures include plant cultivation on substrates (Perelli and Pimpini, 2003). Schmilewski (2008) reported that even though its definition is less precise, the term "substrate" is often used as a synonym for "growing medium". In the US the term "potting media" is more common (Zaccheo and Cattivello, 2009). CEN (1999) defined as growing media all materials that are used to grow plants in substitution of soils. Hence according to this definition growing media include all materials for the professional and hobby markets, indistinctively produced by the substrates industry or home-made and used to grow all types of plants, usually in containers. Growing media constituents, generally formulated on a percentage volume, include several materials (Schmilewski; 2008) Substrates can be divided into organic and inorganic materials. Organic materials include peat (Bilderback, 2001; Cattivello, 2009a), compost (Bilderback, 2001; Centemero, 2009), composted bark, wood fiber, coir dusts, rice hulls and other minor material such as leaves, straw or algae (Bilderback, 2001; Cattivello, 2009b). Inorganic materials are divided into natural mineral components like clays, pumice, sand and zeolites, and heat treated minerals like expanded clay, rock wool, perlite and vermiculite (Cattivello, 2009c). Synthetically produced materials like expanded polystyrene flakes and urea-formaldehyde foam resin are also available (Bunt, 1988; Cattivello, 2009d).

Potting medium quality is of basic importance to obtain good quality plants. Independently of cultivation cycle length, substrates should present suitable physical and chemical characteristics because any mistake made during the seedling or young plant stage is not easily rectified and any problem during this stage of growth will often be evident when the plant is mature (Bunt, 1988).

The main physical properties for substrates have to provide the anchorage that enables the plant to support itself (Bunt, 1988), but with a contemporary low bulk density to obtain a light container and a stable structure to avoid shrinkage (Hanan et al., 1978; Wilson, 1984). Substrates stability, including low shrinkage is required for the optimal growth of plants, especially if the cultivation period is long (Aendekerk, 1997). Another basic physical function of substrates is to regulate water and oxygen supply to the roots (Bunt 1988). Hence substrates should be characterized by good total pore space (not less than 75-80% volume) (De Boodt and Verdonk, 1972) with a suitable repartition between air pore space (10-30%) (Arnold Bik, 1983; Boertje, 1984; Jekins and Jarrel, 1989) and water holding capacity (45-65% volume) to assure proper aeration, water supply and drainage of substrates (De Boodt and Verdonk, 1972; Hanan et al., 1981; Bilderback, 1985; Bilderback et al., 2005). Other physical parameters are available water (25-35%) (Bilderback et al., 2005), easily available water (20-30% volume) and water buffer capacity (4-10% volume) (De Boodt and Verdonk, 1972). Physical properties represent the most important characteristics of substrates because they cannot be modified during plant cultivation (Bibbiani and Pardossi, 2004). Chemical characteristics are: pH that should range from 5.3 to 6.5 (Abad et al., 2001) to maintain nutrients in available forms and avoid loss with precipitation or immobilization (Zaccheo, 2009a); electrical conductivity lower than 0.5 mS<sup>·</sup> cm-1 (Abad et al., 2001); cation exchange capacity and buffer capacity, which are pHrelated characteristics and generally high in organic materials (Zaccheo, 2009a). In order to improve physical and chemical characteristics (e.g. to improve wettability of substrates, to lime peat pH, or to increase nutrient level) media industries usually introduce additives (like fertilizers, liming materials, buffering materials, binders, wetting agents, hydrogels, chemical pesticides, biological products and other substances) to the mix (Schmilewski, 2008; Cattivello, 2009e).

In addition to maintaining their physico-chemical characteristics over time substrates should and resist climatic conditions during cultivation (Bunt 1988), present good microbiological quality and no pathogens (Zaccheo, 2009b), be of uniform quality, available in large quantities and cost effective (Evans et al., 1996; Fecondini et al., 2009).

According to Schmilewski (2008), the quality of a growing medium can be defined in terms of its condition and its suitability for the intended use. Thus the requirements for a specific use determine the quality assignment within that context.

### Peat

Peatlands are peat covered terrain with a minimum peat layer of 30 cm (Rydin and

Jeglum, 2006). Larger peat deposits are in the northern hemisphere in regions with high rainfall and low temperature but smaller ones also occur in subtropical and tropical areas (Bunt, 1988; Cattivello, 2009a). Worral et al. (2003) reported that peatlands form a significant carbon reserve in countries of Western and Northern Europe as well as parts of Canada and Siberia. Peatland extensions and peat occurrence estimations are rather variable. The estimates of the area differ by a factor of two, and those of the total peatland carbon store by a factor of five (Clymo et al., 1998). Gorham (1991) reported that peatlands covered 3.4 million km2 in the boreal and temperate zone and have an estimated carbon store of 455 Gt, as much as approximately one-third of the global soil carbon pool and about 60% of the atmospheric carbon pool. Maltby and Proctor (1996) reported that peatlands cover over 4 million km2 or 3% of the land and freshwater surface of the Earth. Lappalainen (1996) also reported these data but did not include more than 2.4 million km2 of terrestrial wetland ecosystems.

Peat formation started with the end of last ice age 12,000 years ago and it is still ongoing where climatic conditions have favored the development of mires. (Cattivello, 2009a; Orru and Orru, 2008). Peat formation is the result of different factors such as time, temperature, seasonal water balance, nutrient elements availability, position and plant species (Cattivello, 2009a) but also the speed of decomposition of plant residues (Cattivello, 1990). Peat is the final result of partial decomposition of different plant species especially mosses, represented mainly by sphagnum, and vascular herbaceous plants and shrubs in water-saturated environments (Bunt, 1988; Joosten and Clarke, 2002). In conditions such as those of peatlands, the carbon cycle is concluded more slowly than that of other ecosystems and is sometimes interrupted. Hence, lack of oxygen, reduced or interrupted organic matter degradation and mineralization have a consequent positive effect on carbon accumulation. This means increasing partially decomposed organic matter accumulation up to peat formation (Kadlek and Knight, 1996). Peat forms where organic material accumulation is faster than its decomposition and occurs when water supply (rainfall or surface water) is higher than that lost through drainage or evapotranspiration (Cattivello, 2009a). Age-depth profiles show that 10 mm of peat can represent between five and 50 years of accumulation, with typical values for the past 2000 years of 10-20 years per 10 mm (Barber, 1982). The peat formation process is not directly influenced by average temperatures although these affect plant species composition and consequently peat type. There are three mechanisms that permit peat formation: infilling, paludification and primary formation. Peat formation through infilling (or terrestrialization) is the result of progressive organic matter accumulation and sedimentation in a water basin due to plant development. Paludification instead originates peat from substitution of vascular plants forming forests by typical herbaceous and shrub species forming peat. It is the common peat formation of highlands and unlike infilling peat formation there is no sedimentation in the peatland bed. Peat primary formation does not present ponds and sediments and it originates in wet soils (Cattivello, 2009a).

No universal classification system of peat is available, so in some cases it depends on whether it was classified by soil scientists, botanists or horticulturists (Bunt, 1988). Peat classification may be based on:

- condition of peat formation: lowmoor peat, highmoor peat (Bunt, 1988; Perelli, 2003) and blanket bog (Bunt, 1988);
- botanical composition: sedge peat, sphagnum peat (Bunt, 1988; Perelli, 2003) and wood peat (Bunt, 1988; Cattivello, 2009a);
- decomposition grade according to Von Post and Bragg scale (light peat from H1 to H3, dark peat from H4 to H6 and black peat from H7 to H10 (Bunt, 1988; Perelli, 2003);
- nutritional composition: oligotrophic, mesotrophic and eutrophic peat as sphagnum, Phragmites and sedge peat respectively (Bunt, 1988; Perelli, 2003).

Different peat formations are reported but also different types of peatland, such as fens, raised bogs and blanket bogs and tropical peatlands which produce peat with different characteristics. Fens are formed by the shallow flooding of a depression or the infilling of a lake or water basin formed by surface or underground water. The water generally contains some mineral bases especially calcium. Deposits of this type are formed through infilling and may be formed at any altitude, but usually occur at fairly low elevations. Fens originate lowmoor peat and often represent the initial stage for highmoor peat deposits (Bunt, 1988; Cattivello, 2009a). Peat depth in fens is generally 40 cm, and is formed by bryophytes, graminoids and low shrubs. This peat has higher nutrient content and pH (Rydin and

Jeglum, 2006). Raised bogs originate highmoor peat. Peat is formed because the surface is kept continuously saturated by moisture-holding properties of this material and by heavy rainfall and not because of inundation (Bunt, 1988). The peat formation process needs rainfall of between 700-1000 mm per year distributed in 150-175 days (Cattivello, 2009a). It usually overlies lowmoor peat. Highmoor peat is formed in wet conditions and with very low level of mineral bases. Bogs of this type are generally higher in the central part than at the edges, producing a concave shape. They are generally characterized by two layers. The lower one, the older, is constituted of humified peat while the upper one is less decomposed. Material deriving from these layers are generally named 'black peat' and 'white peat' (Bunt, 1988). Highmoor peat is deeper than 40 cm (Rydin and Jeglum, 2006) and is generally 7-8 m thick, with maximum of 13 m, in which the lower layer is 2-3 m thick. (Cattivello, 2009a) Bogs are generally characterized by extremely low nutrient content and pH around 4 (Rydin and Jeglum, 2006). Highmoor peat is generally formed by Eriophorum spp. (Cattivello, 2009) and sphagnum moss (Bunt, 1988; Cattivello, 2009). Blanket bog deposits are similar to highmoor ones, but are formed in areas where the surface is continuously saturated by high rainfall (1250 mm in over 200 days). They largely follow the contours of the ground and the level of mineral bases in the peat is very low, generally 2.5 m. Blanket bogs generally occur on moorland but they can also occur at sea level (Bunt, 1988; Cattivello, 2009a). Although peat generally finds ideal conditions (water availability and low temperatures) for its formation in cool climates, organic matter accumulation can also occur in the tropics, originating tropical peatlands. In this case water availability is promoted by frequent rainfall and soils with poor drainage. Tropical peatlands may also be called peat swamp forest and are generally located on coastal plains and covered by tropical rainforest rather than sphagnum and herbaceous vegetation. Hence peat deriving from this type of peatland is woody and only partially decomposed and is covered by a litter layer. Tropical peatlands are generally from 10 m to 25 m deep and peat formation condition is similar to coal deposits formation millions of years ago. Tropical peatlands are common in south-east Asia (Rydin and Jeglum, 2006).

Peat technical characteristics depend on factors such as botanical composition, decomposition grade, harvest techniques.

Concerning botanical composition peat may be divided into sedge, woody and

sphagnum peat. Sedge peat is formed principally by sedges (Carex spp.), red grass (Pragmites) and some Eriophorum and Calluna plants. Generally sedge peats retain water from 4 to 7 times their dry weight and present a good air pore space thanks to their fibrous structure (Bunt, 1988; Cattivello, 2009a). Sphagnum peat is composed principally by sphagnum moss. There are between 150 and 200 species of the genus Sphagnum (Clymo, 1997) and they are most abundant where acidic, solute-poor water prevails, typically on peat bogs, where each species occupies a habitat range determined largely by the depth of the water table and nutrient availability (Daniels and Eddy, 1990; Clymo, 1997). Because of the morphological structure of sphagnum, which is characterized by a boat-shaped leaf with a single layer cell (Bunt, 1988), peat derived from this moss can retain water from 10 to 14 times its dry weight (Cattivello, 2009a). Woody peats derive from small woody shrubs and are less degradable than sedge peat. They present higher water retention capacity than sedge peat (Bunt, 1988; Cattivello, 2009a).

Decomposition of dead plant parts modifies peat physical and chemical properties. In sphagnum moss for example, leaves detach from the rachis reducing total pore space and air pore space and increasing bulk density, water holding capacity, cation exchange capacity and buffer capacity (Cattivello, 2009a).

Peat harvesting methods also influence peat technical characteristics. Peat harvest starts by opening a drainage system in the peatland to reduce moisture content to permit access to operating machines. Three methods are known for peat harvesting: milling, sod cutting and hydraulic mining. Milling is generally used with peat that presents a low degradation level ( $\leq$  H5). Prior to harvest, peat is milled, dried to 60-65% of moisture content and consequently harvested with collecting or vacuum machines. As an alternative to milling, for the same class of decomposition, peat may be collected using sod cutting in which peat is cut and laid out in brick-shaped units. This harvesting method is used to obtain peat with a higher air volume, but is more expensive. Peat with H6 or higher degree of decomposition is harvested by hydraulic mining. In this case peat, collected in cylinder-shaped units in autumn, is subjected to ice action during winter that improves physical properties and will be harvested during the subsequent summer after drying. Peat harvested with this method is generally used in mixes with milled peat to produce growing media with fine or medium-fine particle size (Bunt, 1988; Cattivello, 2009).

Concerning the nutritional composition of peat, oligotrophic peat is extremely low in nutrients and biological activity and its organic matter is little decomposed as sphagnum peat. Mesotrophic peat presents a mediocre or moderate nutrient availability and slow organic matter decomposition, while eutrophic peat is rich in nutrients (Bunt, 1988).

The chemical composition of peat depends on feeding type, geomorphological position, vegetation, geological, geobotanical, and microbiological processes (Orru and Orru, 2008). It presents a minimum 30% organic matter content (Bunt, 1988; Joosten and Clarke, 2002). Its organic part is a mixture of plant remains at different stages of humification. The organic constituents of peat can be classified into bitumens, carbohydrates, lignins and humic substances (Fuchsman 1980). Besides these, peat contains nitrogen components, inorganic substances, etc. Peat is well humified if over 25% of its organic mass has decayed, and poorly humified when the decayed constituents form less than 25%. Peat dry matter can contain up to 35% of minerals. Peat is organic matter accumulating on the surface of the ground and containing a high proportion of water (92-94%) (Orru and Orru, 2008).

#### Peat exploitation problems and possible alternatives

Thanks to its desirable physical and chemical characteristics like low bulk density, high water-holding capacity, good aeration porosity, low soluble salts, acceptable pH, and high uniformity across batches, peat is the material most widely used alone or mixed with other materials (Schmilewski, 1983; Raviv et al., 1986; Bunt, 1988; Stamps and Evans, 1999; Abad et al., 2001; Ostos et al., 2007; Schmilewski, 2008; Cattivello, 2009a). Sphagnum peat has been the standard base component for most container growing substrates since the 1950s (Krucker et al., 2010). Clarke (2008) reported that peat is considered an essential material for horticulture because it is the only substrate available in industrial amounts, because peat alternatives can be only added to substrates and not totally substitute peat and because no alternative material to peat exists that is available in sufficient quantities to offer the same uniformity and quality to the grower. A study conducted in the EU revealed that in 2007 peat represented 84% of all materials used for substrates production with a total amount of 26 million m3 and that this increased slightly in 2008 to 86%, for a total of 26.8 million m3. In the same study the author reported that

Italy resulted as being the major peat consumer in the EU (Altmann, 2008).

Peat is the most widely used growing media constituent for potted plants production, but since the 1970s there has been a worldwide search for new peat substitutes (Raviv et al., 1986; Robertson, 1993). The reasons for the search for alternatives are linked to the high price for high quality horticultural peat, especially in countries without peat resources. Other reasons are the availability of peat because it is considered a non-renewable resource and environmental constraints due to its exploitation (Hadar et al., 1985; Raviv et al., 1986; Verdock, 1988). There are several reasons for protecting the peatlands, such as preservation of biological and landscape diversity, preservation of natural resources (clean water, berries, herbs), as a habitat for threatened species, scientific value as a reference area for its uniqueness or typicality, recreational and educational value for humans and ecotourism (Orru and Orru, 2008). Moreover, Barber (1993) reported that peatlands are the most vulnerable of all natural ecosystems to irreversible damage. In addition to this peatlands are important sinks and sources of carbon. (Worral et al., 2003). For these reasons the EU and national law have adopted measures limiting peat exploitation that have caused reducing availability of this material (Gallagher, 2008).

Recent research has sought to identify alternatives to traditional peat, focusing on reusable, recyclable materials not derived from non-renewable sources such as peat bogs (Hadar et al., 1985; Raviv et al., 1986; Verdock, 1988). Under this aspect, agro-industrial activities produce high quantities of biomasses that in some cases can be used as ingredients for growing media production, re-cycling materials that would otherwise be considered waste to be disposed of (Grigatti et al., 2007; Ostos et al., 2007).

A number of studies have shown that organic residues such as urban solid wastes, sewage sludge, pruning waste, spent mushroom and even green wastes, after proper composting, can be used with very good results as growth media instead of peat (Siminis and Manios, 1990; Pryce, 1991; García-Gómez et al., 2002; Benito et al., 2005).

#### **Rice hulls**

Rice hulls (or rice husks) are the protecting structure that covers seeds of the rice plant (Yat et al., 2008), and that is separated from the grains during the rice milling process (Cattivello, 2009b). Rice hulls are yellow or light brown structures and are made of hard

and fibrous materials like hemicellulose lignin, cutin, opaline and silica (Juliano et al., 1987; Ma et al., 2011).

The rice hull represents 17 to 23% of dry weight of the harvested rice (Cattivello, 2009b; Ma et al., 2011) and since rice is one of the major crops in the world (FAO, 2009) this by-product is available in large volumes (Savita and Kamath, 1998). Countries of Asia are the biggest producers of various rice products in the world (Ma et al., 2011) but they are also readily available in the USA (Kamath and Proctor, 1998) and in Italy (Sambo et al., 2008). Nowadays the rice by-product can be used in different ways. Rice hulls can be used for liquid fuel production thanks to high cellulose content to produce ethanol (Ma et al., 2011). Rice hulls are also burned to produce energy (Kalapathy et al., 2000). Rice hull ashes are very rich in silica so can be used to produce silica gel (Kamath and Proctor, 1998). Another study revealed that this element may be useful to reduce Phytium dampingoff in cucumber plants (Jeffrey et al., 1997). Rice hulls are also used for poultry litter (Kelleher et al. 2002). Finally rice hulls are often used as an ingredient in growing media for horticultural production since they present some interesting physical characteristics (Zanin et al., 2011). Unfortunately only 10% of rice hulls generally end up as a byproduct, which means there is excessive waste (Ma et al., 2011) and although various uses for rice hulls have been suggested, their disposal or utilization remains a major concern (Kalapathy et al., 2000).

Considering the wide use of peat for growing media production the use of rice hulls in substrates may be useful to dispose this by-product and to reduce peatland exploitation obtaining cheaper substrates (Einert, 1972; D'Angelo et al., 1993). For this reason, the physical, chemical and agronomical aspects of rice hulls have been studied since the 1970s.

Fresh rice hull is characterized by bulk density and total pore space similar to medium decomposed peat (Cattivello, 2009b). Whole fresh rice hull presents large particle size, so its use may be to improve air pore space and drainage in a substrate (Evans and Gachukia, 2007), but also poor capillarity. For this reason it is not suggested to use this material alone to avoid not uniform moisture content in the growing media. It also presents good stability (Cattivello, 2009b). In order to improve water holding capacity and capillarity, partial rice hull carbonization was studied. This process improved substrate wettability because it removed the external wax layer of rice hull (Calderon, 2001). Kampf

and Jung (1991) observed that completely carbonized rice hulls increased air porosity and bulk density of substrates reducing water holding capacity. Water holding capacity in composted rice hulls resulted as being higher than fresh or carbonized but was still lower than advisable (Garcia-C. et al., 2001). Rice hull grinding was demonstrated to be a suitable method to improve its physical properties that resulted much closer to those of peat (Sambo et al., 2008; Buck and Evans, 2010).

Under the chemical aspect rice hull pH was demonstrated to be neutral (Zanin, 2011) also after carbonization process (Kampf and Jung, 1991) and this element may be useful to correct peat substrate values (Zanin, 2011). Rice hull is characterized by fair cation exchange capacity. Among nutrients this material is rich in phosphorus, potassium, silica and manganese (Cattivello, 2009b), but the last element may be toxic with pH values lower than 5.0 (Einert, 1972; Evans and Gachukia, 2008; Cattivello, 2009b). Rice hull addition to peat substrates reduced calcium, magnesium and iron content (Gachukia and Evans, 2008).

Rice hull use in substrates requires some expedients. As it is a material characterized by high C/N ratio, some problem may arise in nitrogen availability (Perelli and Pimpini, 2003), but no nitrogen depletion occurred in a study conducted by Evans and Gachukia (2004). However, if cultivation exceeds 6 months higher nitrogen amounts are required to balance the ratio that is used by microorganisms in the decomposition process (Cattivello, 2009b). Rice parboiling process exposes rice hulls to steam at 100–130 °C (Derycke et al., 2005) so parboiled rice hulls use is recommended to avoid soil-borne diseases, rice seed germination and release of toxic levels of Mn (Einert, 1972; Cattivello, 2009b).

Under the agronomical aspect rice hull suitability has been demonstrated as partial peat substitute. Higher air pore space increased root development but higher watering is required in summertime (Einert, 1972; Einert and Baker; 1973). Both fresh and composted rice hulls can partially substitute vermiculite in *Impatiens walleriana* cultivation (Dueitt et al., 1993). Rice hulls can also efficiently substitute perlite. *Pinus halepensis* growth in substrates with rice hulls resulted as equal to or better than plants grown on perlite (Tskaldimi, 2006). Fresh and parboiled rice hulls represent a reliable perlite alternative for growing herbaceous ornamentals (Papafotiou et al., 2001; Evans and Gachukia, 2004). In

particular, Evans and Gachukia (2004) outlined that plant growth of tomato, geranium, pansy, marigold, impatiens and vinca was comparable in substrates where perlite was replaced with an equivalent amount of fresh rice hulls. Herbaceous and woody ornamental plants grown in substrates with rice hulls presented good growth (Laiche and Nash, 1990; Garcia-C. et al., 2001; Marianthi, 2006). Vegetable cultivation resulted possible for tomato and pepper production without yield and quality losses for tomato (Snyder, 1994), but Del Amor and Gómez-López (2009) revealed that more caution is required is required for pepper cultivation. Calderon (2001) reported that fresh rice hulls were commonly used for hydroponic cultivation in South America. Zanin et al. (2011) used increasing ground rice hull content in substrates to grow vegetable seedlings, obtaining good results for chicory and decreasing quality in tomato and pepper.

Considering these results whole rice hull should not exceed 20-30% by volume in substrates, even though in some cases higher percentages (up to 50%) can be used (Cattivello, 2009b).

#### Anaerobic digestion residues

Anaerobic digestion is the organic matter decomposition operated by a microbial consortium in an oxygen-free environment (Pain and Hepherd, 1985). This process occurs naturally in many anoxic environments such as watercourses, sediments, waterlogged soils and the mammalian gut (Ward et al., 2008). In recent years the interest in anaerobic digestion has grown, with an increasing number of anaerobic treatment plants (Hansen et al., 1999; Tani et al., 2006) and it can be applied to a wide range of organic materials including industrial and municipal wastewaters, agricultural, municipal, food industry wastes, and plant residues (Ward et al., 2008). In this process organic matter is transformed into biogas which principally consists of methane (50–80% v/v), carbon dioxide 36–41%, up to 17% nitrogen, less than 1% oxygen, 32–169 ppm hydrogen sulphide and traces of other gases (Tambone et al., 2008). In addition to gas this process produce digestate, a residual organic matrix (Ward et al., 2008) that is often dewatered to reduce the volume and mass for transport. During this process solid and liquid separation is operated using belt filter presses, vacuum filtration, or centrifugation so that the material takes on the

properties of a solid. Its final moisture content generally varies from 60 to 75%. It can also be composted to further reduce volume, produce a more stabilized product, and reduce the incidence of pathogens (Haynes et al., 2009).

Anaerobic digestion of organic material is an environmentally beneficial practice and offers significant advantages. In fact this process:

- contains the decomposition processes in a sealed environment, preventing potential damage from methane entering the atmosphere by burning the gas (the carbon dioxide produced burning methane is carbon-neutral backing to the carbon cycle),
- the energy gained from combustion of methane will displace fossil fuels, reducing the production of carbon dioxide that is not part of the recent carbon cycle,
- reduces the biomass sludge in comparison to aerobic treatment technologies,
- is successful in treating wet wastes with less than 40% dry matter,
- can remove pathogens, especially in multi-stage digesters or if a pasteurization step is included in the process,
- remarkably reduces odor emissions,
- reduces greenhouse gas emissions (Amon et al., 2006),
- reduces the amount of biodegradable waste entering landfill (Ward et al., 2008).

High quantities of organic wastes are produced by agro-industries and these biomasses are typically rich in nutrients and can be used in agriculture to conserve and recycle nutrients, to reduce waste discharge and use of chemical fertilizers as organic amendments and/or fertilizers (Salminen et al., 2001; Tambone et al., 2008; Holm-Nielsen et al., 2009). During the anaerobic process, waste physical and chemical properties are improved and its potential phytotoxicity is reduced. For example, the major part of organic nitrogen is converted to ammonia (Salminen et al., 2001) and the final product presents a low C/N ratio, generally from 10 to 20, and high nutrient levels (Salminen et al., 2001; Tambone et al., 2008; Haynes et al., 2009). Biosolids samples are typically made up of 40–70% organic matter (measured by loss of mass on ignition). Digestates generally have an organic carbon content ranging from 20 to 50% and total nitrogen content from 2 to 5%

(Haynes et al., 2009).

Some studies were aimed to investigate nitrogen, methane and carbon dioxide losses of digestate (Amon et al., 2006; Hou et al., 2007; Grigatti et al., 2011).

Schröder et al. (2007) calculated the nitrogen fertilizer replacement value (NFRV) of digested and not digested liquid manures compared to mineral fertilization in a permanent grassland. In this study NFRV values of digested manure were similar to those of not digested. Similar results were found in another study on grassland and on arable land comprising a whole crop rotation with cereals and potatoes. Also in this case, no differences were observed between two digestate biomasses on biomass yield and nitrogen availability (Möller et al., 2008).

The solid fraction of anaerobic digestion residues deriving from cattle manure was used to grow Cypripedium orchids. Plants obtained in substrates with digested material were similar to those grown on coconut coir (Compton and Zauche, 2006a). The same authors used the same anaerobic digestion residue to grow *Geranium* ×*hortorum* 'Red Elite' in peat substrates. In this case plants grown on substrates with digested biomass presented better values than those grown in peat (Compton and Zauche, 2006b).

Anaerobic digestion residues deriving from plant biomasses can also be used. Pomace digested materials were used to fertilize a vineyard (Cantagrel et al., 1990). Sambo et al. (2010) obtained good results fertilizing lettuce with anaerobic digestion residues of fruit but in association with mineral fertilization. Unfortunately little information is available about the use of anaerobic digested residues derived from plant byproducts (ADR) as fertilizer in substrates. In table 1 is reported chemical characterization of anaerobic digestion residues used in this study.

	Chapter				
	$2^{nd}$	3 <sup>rd</sup>	$4^{th}$ and $5^{th}$	6 <sup>th</sup>	
pH	7.68	8.12	8.37	7.83	
Electrical conductivity (mS·cm <sup>-1</sup> )	1.46	1.27	1.07	1.12	
Cation exchange capacity (meq '100g)	76.5	78.2	86.9	79.3	
Organic matter (%)	49.9	57.8	41.2	60.7	
Organic carbon (%)	29.0	33.5	23.9	35.2	
Dry matter (%)	30.2	30.0	34.6	28.9	
Total kjeldhal nitrogen (%)	3.90	3.70	2.66	3.95	
C/N ratio	7.43	9.05	8.97	8.91	
$N-NO_3 (mg \cdot L^{-1})$	30.3	31.9	0.13	0.03	
$P(mg \cdot L^{-1})$	6.95	31.0	6.07	7.74	
$K (mg \cdot L^{-1})$	317	149	160	171	
$Ca (mg \cdot L^{-1})$	21.8	21.1	9.26	7.13	
$Mg (mg \cdot L^{-1})$	2.39	5.51	1.02	0.73	

Table 1. Chemical characterization of anaerobic digestion residues used in this study

# **Objectives of the study**

Nowadays, increasing peat prices related to higher transport costs and to stronger limitation of peat exploitation, but also to higher energy costs, have raised production costs of containerized nursery and horticultural production. In addition, agriculture, like other human activities, is now called upon to adopt a more sustainable approach. The horticultural sector, which traditionally uses large amounts of inputs like water, peat and fertilizers to assure maximum quantities and qualities in seedling, rooted cutting and potted plant production, is also involved. For this reason producers are looking for cheaper alternative materials to peat available in good quantities and that maintain production quality. Agro-industrial activities produce great quantities of biomasses that in many cases are considered waste and have to be disposed of. An interesting solution may be to recycle those materials, after proper treatments if needed, as a component in substrates or as fertilizer, meeting the need to reduce peat use and to exploit new potential resources, reducing environmental impact.

The overall objective of this study was to investigate the possibility to partly or totally substitute peat with rice hulls and evaluate the effect of anaerobic digestion residues of plant origin used as fertilizer in substrates for different nursery and horticultural productions. In particular, after their physical and chemical characterization, the study aimed to investigate the effect of different substrates on vegetable and ornamental seedlings, for rooting ornamental cuttings, for cultivating potted ornamentals and for rooting and cultivating small ornamental cuttings without transplant.

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**Chapter II** 

Rice hull-based substrates fertilized with anaerobic digestion residues for vegetable and flower transplant production

## Abstract

The widespread exploitation of peat has caused environmental issues and increasing prices, so researchers are focused on finding alternative materials. Agricultural byproducts and organic wastes may be used only if they present good physical and chemical characteristics. Rice hulls have been studied by several authors, who demonstrated that they can be suitable for producing growing mixes. Less is known about the use of anaerobic digestion residues (ADR) in growing media. Different combinations of sphagnum peat and ground rice hulls (GRH) (0, 33, 67 and 100% by volume of GRH) were studied. ADR (20% by volume), considered as fertilizer, were added or not to substrates. Substrates were physically and chemically characterized and then tested as substrates for transplant production of tomato, Salvia officinalis, Salvia splendens and Begonia semperflorens in two experiments aimed to investigate differences between species (experiment 1) and over time (experiment 2). Physical properties were affected by both GRH percentage and ADR addition. GRH generally decreased parameters regarding water holding capacity, while ADR limited this phenomenon only in substrates with 0 and 33% GRH. Chemical analysis pointed out a possible nutrient imbalance due to increasing GRH ratio in the media because of increased P and K in the water extract, while ADR generally increased nutrient content in substrates. In experiment 1, the increase of GRH in the mix without ADR negatively affected growth of S. splendens and B. semperflorens in particular. In general, when the rate of GRH was higher than 33%, seedlings did not reach marketable size at the same time as the peat control. The addition of ADR improved the characteristics of all mixes. In experiment 2, tomato, S. officinalis and S. splendens plants showed few or no differences between plants that reached marketable size first. S. splendens needed a longer time than the other species to reach marketable size. B. semperflorens never reached an acceptable standard when substrates with GRH were used. This was probably due to the ornamental species having higher sensitivity to water holding capacity and easily available water reduction than tomato and S. officinalis.

### Introduction

Peat represents the most important ingredient for growing media production. In 2006 the peat volume consumed in Europe to produce root substrates was 77.4% of total

volume of material used (Schmilewski, 2007) and it is of great importance for vegetable transplant production (Gruda and Schnitzler, 2004). Nowadays the wide use of peat is going to be limited by some economic and environmental issues. Indeed the rising price of this material in southern European countries that import peat (Ribeiro et al., 2007) and the increasing interest in wetland conservation (Barkham, 1993; Robertson, 1993) have led to an interest in looking for alternative materials (Ribeiro et al., 2007).

Peat substitutes in growing media must be uniform in quality, available in great quantities, cost effective and have suitable physical and chemical properties (Evans et al., 1996; Fecondini et al., 2009). Since no universal substrate exists, many are constituted by a mixture of peat with other material in order to achieve desirable physical and chemical properties (Bunt, 1971; Fonteno, 1993; Bachman and Metzger, 2007).

Some peat alternatives, recycling various organic residues generated by agriculture and agro-industries and human activities, were successfully used as container media. For instance Arenas et al. (2002) tested some substrates with peat, coir, vermiculite or perlite to determine the optimum growing media for tomato and outlined that coir should not exceed 50% by volume with peat in substrates to avoid reduction of plant growth. Gruda and Schnitzler (2004a) found that the physical properties of some substrates were modified by the addition of wood fiber in substitution of peat and demonstrated their suitability for tomato transplant production (Gruda and Schnitzler, 2004b). In another study, tomato transplants grown in a substrate with 30% by volume of municipal solid waste compost did not differ from those grown in a peat control (Herrera et al., 2008). The suitability of compost deriving from forest wastes and solid phase of pig slurry as partial alternative to peat was demonstrated for tomato and lettuce seedlings (Ribeiro et al., 2007). Spent mushroom substrates modified the chemical and physical properties of substrates for seedling production and its use can substitute up to 75% by volume of peat to grow tomato, courgette and pepper seedlings (Medina et al., 2009). In another study, the physical and chemical properties of substrates with composted sewage sludge appeared comparable with those of the peat control and adequate for broccoli propagation (Perez-Murcia et al., 2006).

Rice hulls are an interesting rice byproduct that can be used as a component of substrates and are readily available in large volumes (Del Amor and Gomez-Lopez, 2009). Some studies revealed that carbonized rice hulls can improve peat physical properties,

increasing air porosity (Kampf and Jung, 1991), while water holding capacity (WHC) in composted rice hulls is higher than in the fresh material but is still lower than advisable (Garcia-C. et al., 2001). Rice hulls in growing media mix allow good plant growth (Laiche and Nash, 1990; Garcia-C. et al., 2001). Good results were observed growing some ornamental plants on growing media with fresh and parboiled rice hulls as perlite alternative (Papafotiou et al., 2001; Evans and Gachukia, 2004). The use of parboiled rice hulls is suggested to destroy pathogens, rice seeds and avoid the release of toxic levels of Mn (Einert, 1972). Recently physical features much closer to those of peat were observed in some experiments with ground fresh rice hulls (Sambo et al., 2008; Buck and Evans, 2010).

Anaerobic digestion is a process that decomposes organic matter and it can be applied to a wide range of biomasses (e.g. plant residues, sewage sludge, the organic fraction of municipal wastes, agricultural byproducts) (Ward et al., 2008) in which the final residues generally present low C/N ratio and high nutrient levels. For these reasons digestate may be used in agriculture as both fertilizer and amendment (Salminen et al., 2001; Tambone et al., 2008) after separation into a liquid and a solid fraction (Ward et al., 2008). Good results were obtained using anaerobic digestion biosolids derived from cattle manure in substrates to grow ornamental plants (Compton and Zauche, 2006a; Compton and Zauche, 2006b). However little is known about the use of anaerobic digested residues (ADR) derived from plant byproducts as fertilizer in substrates for seedling production.

The aim of this study was to evaluate the chemical, physical and agronomic properties of some substrates containing ground rice hulls (GRH) and ADR as partial component of substrates for vegetable transplant production to try and reduce the amount of peat-based substrates.

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### Material and Methods

#### Substrates preparation

Four substrates (0-, 33-, 67- and 100-) were prepared by mixing 2 mm fresh ground rice hulls (GRH) with a peat-based commercial substrate for transplant production. GRH percentage on substrates was 0, 33, 67 and 100% by volume respectively. Four more substrates (0+, 33+, 67+ and 100+) were prepared with the same proportion and fertilized with 20% by volume of ADR deriving from fruit distillery wastes.

### Physical properties of substrates

Bulk density (BD) was determined adopting the EN 13040 methodology (1999). Water retention curves were performed on five samples of each of the 8 substrates and were developed using a pressure plate system in which samples were saturated with deionized and deaerated water (Tempe cell 1400, Soil Moisture Equipment Corporation, Santa Barbara, USA) and subjected to increasing pressures of -1, -2, -5 and -10 kPa. In order to determine the volume of water retained at each pressure sample weights were recorded when pressure systems reached equilibrium status. The sum of all the water held on the tempe cell was considered total pore space (TPS) (Fonteno and Bilderback, 1993). Water volume held at pressure between 0 and -1 kPa was considered air-filled porosity (AFP). Water at -1 kPa was considered as water-holding capacity (WHC). Water held at pressures between -1 and -10 kPa, between -1 and -5 kPa and between -5 and -10 kPa were considered as available water (AW), easily available water (EAW) (Bunt, 1988; De Boodt and Verdonck, 1972) and water buffering capacity (WBC) respectively (Bruckner, 1997). Water released at pressure higher than 10 kPa was considered unavailable water (UW) (Ingram et al., 1993). The AW and EAW were then divided by the WHC to obtain available water percentage (AW%) and easily available water percentage (EAW%) (Sambo et al., 2008).

#### Chemical properties of substrates

pH and electrical conductivity (EC) were investigated using EN 13037 and EN 13038 methodologies, respectively. EN 13039 was used to determine organic carbon (OC)

and organic matter (OM) content. Cation exchange capacity (CEC) was determined using BaCl2-triethanolamine (Lax et al., 1986). Total Kjeldahl nitrogen (TKN) was also measured. Nitrate-nitrogen (NO3-N), P, K, Ca, Mg and Mn were extracted with deionized water according to EN 13652 methodology. Water extraction was preferred to other official methods (e.g. EN 13651) because it gives a measure of nutrients promptly available to plants even if it under-estimates their total content. NO3-N was evaluated by means of ionic chromatography (ICS-900, Dionex, Sunnyvale, CA, USA) and P, K, Ca, Mg and Mn contents were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES with SPECTRO CirOS Vision EOP, SPECTRO Analytical Instruments GmbH & Co. KG, Kleve, D). Concentrations of Al, Cd, Cr, Cu, Hg, Ni, Pb, Zn were also determined in the dry matter adopting the Zancan et al. (2006) procedure for mineralization and ICP procedure for reading.

### Agronomic evaluation of substrates – experiment 1

Vegetable and flower transplants were cultivated in a PE film greenhouse with openings in the roof and at the sides. Seedlings of tomato 'Jack', *Salvia officinalis*, *Salvia splendens* 'Maestro' and *Begonia semperflorens* 'Super Olympia Red' were grown in plastic trays with 300 cells (4000 mm<sup>3</sup> volume each). In the same tray substrates with different ratio of GRH and peat were evaluated with or without ADR (144 cells per treatment). Trays were hand-sown, covered with a vermiculite layer, watered and moved to the greenhouse. Irrigation was applied as needed by each substrate according to the "look and feel" method (Niederholzer and Long, 1998) and Raviv and Lieth (2007). Starting from 10 days after sowing, when plants developed the first true leaf, fertigations were applied 3 times per week using a 13-5-20 (N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O) plus microelements hydro-soluble fertilizer. Electrical conductivity of the nutrient solution was raised during the cropping cycle from 0.8 to  $1.2 \text{ mS cm}^{-1}$ .

Regardless of treatments, when seedlings reached the commercial standard at least in one treatment, 10 plants for each species, treatment and block were sampled. Samplings occurred 28 days after sowing tomato and *S. splendens*, 45 for *S. officinalis* and 65 for *B. semperflorens*. Seedling height, number of true leaves, stem diameter, hypocotyl length, fresh and dry weight and dry matter of aerial part were evaluated on tomato and both salvia species seedlings. Seedling height, number of true leaves, fresh and dry weight and dry matter of aerial part were evaluated on B. semperflorens seedlings.

### Agronomic evaluation of substrates – experiment 2

These evaluations were repeated at the achievement of commercial standard for each treatment and additional days to complete the cultivation cycle were recorded.

### Data statistical analysis

Each species' dataset was analyzed by analysis of variance (ANOVA) and means separated according to Tukey's HSD test ( $P \le 0.05$ ). Data on physical-chemical characterization of substrates were analyzed as a factorial experiment in a two-way completely randomized design, while a split-plot design with four replications (16 trays per species) was adopted for the cultivation trial. Values expressed as percentages were transformed prior to ANOVA analysis.

## **Results and Discussion**

### Physical properties of substrates

Bulk density (BD) of substrates (Tab. 1) ranged from 0.264 to 0.377 g cm<sup>-3</sup>, and was affected by the interaction between the two substrate components. Substrates 0-, 33- and 67- had few differences while 100- had a significantly higher BD of 12.4%. Addition of ADR increased the BD of growing media (plus 28.4%) but a progressive reduction of values was observed with the increase in the rate of GRH. The different trend observed between ADR-fertilized and ADR-free substrates could be due to issues concerning settling and packing of particles which, as reported by other authors (Buck and Evans, 2010), are less predictable when three components rather than two are mixed in a substrate. Even if BD values were slightly higher than recommended for transplants (0.06-0.25 g cm<sup>-3</sup>) (Aendekerk et al., 2000), they are in the range proposed by Abad et al. (2001) (0.40 g cm<sup>-3</sup>) and by Yeager et al. (2007) for containerized crop growing media (0.19-0.70 g cm<sup>-3</sup>).

TPS (Tab. 1) was affected only by GRH percentage on substrate. Values initially decreased from 89.1% (0% GRH) to 67.2% (67% GRH) and then increased up to 78.7% (100% GRH). Even if only 0% GRH substrates presented optimal TPS values (De Boodt and Verdonck, 1972), according to several authors (Boertje, 1984; Jenkins and Jarrell, 1989; Crippa, 2009) all substrates presented acceptable values (between 60 and 95%).

Interaction for AFP (Tab. 1) was significant and addition of ADR with 0% GRH significantly decreased its value while with 100% increased its value; at 33 and 67% GRH no differences were found between treatments with or without ADR. This apparently strange response (initial decrease and subsequent increase) is, of course, in part related to that of BD. The lowest values of AFP were found with 33% GRH which are in the ideal range for substrates (10-30%) (De Boodt and Verdonck, 1972; Raviv et al. 2002) while 0+, 67- and 67+ were slightly higher. Other values were too high but are a result of the experimental treatment, as rice hulls have higher AFP.

WHC (Tab. 1) was reduced by the increase of GRH in the substrates (from 51.2% with 0 GRH to 28.1% with 100 GRH. This reduction was more evident when ADR was added to the substrate. According to Boertje (1984), substrates with 0 and 33% GRH had optimal WHC (45%) while substrates with 67 and 100% GRH presented lower values.

AW and EAW (Tab. 1) presented the same decreasing trend of WHC values increasing GRH content, with a reduction of 67.7% and of 71.5% with 0% and 100% GRH respectively. As the optimal range for EAW is 20-30% all substrates presented low values (De Boodt and Verdonck, 1972). However the substrates with characteristics closer to the recommended values are those without GRH. WBC of the substrates was only affected by the rate of GRH and values were higher in GRH-free substrates (on average 4.1%) than in all the others (on average 2.1%). Again substrates with 0% GRH presented optimal values for WBC, while GRH reduced this parameter (De Boodt and Verdonck, 1972). According to Benito et al. (2006), low values of EAW and WBC suggest that water should be applied frequently and in small quantities.

UW (Tab. 1) decreased slowly (14.7%) with increasing GRH content on AFR-free substrates. Addition of ADR significantly raised UW content on 0% GRH substrates (+34.7%).

### Chemical properties of substrates

The analysis of variance performed on chemical characteristics in some cases indicated an interaction effect between the two factors and these results are reported in table 2. Where interaction was not significant data are reported in table 3.

Substrates without ADR increased pH values (Tab. 2) from 5.69 to 6.27 as GRH increased. As ADR presented basic pH, the addition of this material to the substrate influenced pH, raising values in substrates, but the pH decreased as the rate of GRH increased, probably because of increased buffering capacity. This may also be related to the decreasing CEC (Cattivello, 2009). According to Carlson and Rowley (1980), pH values were within optimal range for bedding plants production (5.5-7-0) even if 0+ presented a neutral value.

Considering the interaction GRH x ADR, the cation exchange capacity (CEC) (Tab. 2) decreased from 146 to 33 meq  $100g^{-1}$  with the increase of GRH percentage in the growing medium. ADR addition reduced the control CEC by 20.5%. No significant differences were observed between substrates with 33% and 100% GRH, while CEC of 67-was 21.7% lower than 67+. Even if the control CEC was lower than that of commercial peat-based substrates analyzed by Benito et al. (2006), CEC values were higher than the range suggested by Cattivello (2009). This is probably due to the effect of high organic

matter content in the substrates (Benito et al., 2006; Nagase and Dunnett, in press 2011).

EC values (Tab. 2) increased when the rate of GRH was above 33%, while substrates that were GRH-free had a higher EC than 33% and 67% GRH substrates (Tab. 3). This is probably due to a BD effect. Substrates containing ADR had higher EC values with differences between ADR+ and ADR– substrates decreasing as the rate of GRH increased. However, all values were lower than 0.5 mS cm<sup>-1</sup>, thus within an acceptable range (0.2-0.5 mS cm<sup>-1</sup>) for plant growth (Abad et al., 2001; Pozzi and Valagussa, 2009).

TKN percentage (Tab. 2) decreased by 46.7% as the rate of GRH increased. In general ADR-fertilized substrates presented 2.46 times higher TKN with respect to those without ADR and with a high rate of reduction compared to ADR-free substrates. Control TKN content was slightly lower than the TKN content suggested by Bunt (1988) (1-2.5%).

Even though a statistical analysis was not possible on the C/N ratio because of the independence of samples, values were much higher in ADR-fertilized than in ADR-free substrates. Furthermore, values increased along with the increase of percentage of GRH (Tab. 2). As the C/N ratio recommended by some authors (Abad et al., 1992) is 20-40%, ADR-fertilized substrates appeared to be more suitable for plant growth than ADR-free substrates.

In substrates without ADR, P concentrations (Tab. 2) rose from 1.71 to 16.6 mg L<sup>-1</sup> with the increasing of GRH percentage. Substrates with ADR presented a higher level for this nutrient (on average 17.9 mg L<sup>-1</sup>), but the concentration was not affected by the different GRH content. According to Pozzi and Valagussa (2009), only 0- and 33-substrates had values in the normal range (6.11-8.29 mg L<sup>-1</sup>). P concentration of the control was similar to sphagnum peat used by Evans et al. (2011). Converting P concentration into P<sub>2</sub>O<sub>5</sub> percentage, values are similar to those found by Iranzo et al. (2004) on rice straw.

Also K concentration (Tab. 2) in substrates not fertilized with ADR increased (from 5.65 to 115.7 mg  $L^{-1}$ ) with the increasing of GRH content. Substrates with ADR showed 28.6% higher K concentration than ADR free substrates, with little difference between them. K concentration of 0- was the only one in the normal range (4-14 mg  $L^{-1}$ ) reported by Pozzi and Valagussa (2009) and it is similar to data obtained by Evans et al. (2011). High levels of P and K in substrates is due to the presence of rice hulls, particularly if ground (Cadell, 1988; Cattivello, 2009; Zanin et al., 2011).

Concentrations of Ca (Tab. 2) in ADR-free substrates decreased by 67.4% when the rate of GRH was higher than 33% (Table 3). Ca concentrations were higher in ADR-fertilized substrates and rose with the increase of GRH but only up to 67%. Furthermore, the latter substrates had acceptable values (within 10-19 mg  $L^{-1}$ ), while the former had low concentrations (Pozzi and Valagussa, 2009).

Mn concentrations (Tab. 2) increased along with the increase of GRH, being higher in the absence of ADR. According to Pozzi and Valagussa (2009), values were already higher than recommended (0.01-0.1 mg  $L^{-1}$ ) with 33% GRH. High values of Mn are known in GRH (Cadell, 1988; Evans and Gachukia, 2008; Cattivello, 2009).

OM and OC (Tab. 3) increased from 71.4 to 80.8% and from 41.4 to 46.7%, respectively, as GRH in the substrate increased from 0 to 67%. With a further increase of GRH no significant changes were observed. The addition of ADR to the substrates reduced OM and OC values. According to Abad et al. (2001), OM on substrates with 67 and 100% GRH appeared in the proper percentage ( $\geq$ 80%). Moreover OM of GRH was similar to that observed by Zanin et al. (2011). Substrates with 0 and 33% GRH had a lower than advisable OM percentage (Raviv et al., 2002; Abad et al., 2001) probably because the starting material was a commercial peat-based substrate, and not pure peat.

NO<sub>3</sub>-N concentration (Tab. 3) in the water extract was affected only by the rate of GRH. In fact values decreased from 29.9 to 1.0 mg L<sup>-1</sup>. Values appeared remarkably low if compared with value range proposed by Abad et al., (1992) but slightly higher than NO<sub>3</sub>-N concentration of peat tested by Larcher and Scariot (2009) and Benito et al. (2006). Substrates containing 33% GRH or less had normal values (11-23 mg L<sup>-1</sup>) for a growing substrate (Pozzi and Valagussa, 2009).

Substrates with 100% GRH presented lower values of Mg concentrations in the water extracts (Tab. 3). ADR-fertilized substrates presented 16.9% higher Mg concentrations. In all substrates values were in accordance with the normal values (6-10 mg  $L^{-1}$ ) proposed by Pozzi and Valagussa (2009).

The concentrations of heavy metals in the ADR-containing substrates were higher than in ADR-free substrates, but decreased along with the increase in GRH percentage in the substrates (Tabs. 2 and 3). However all values were much lower than values proposed by Abad (1992) and the more restrictive Italian legal limits (Decreto legislativo 29 aprile 2010, n. 75). Moreover, the levels of Cd and Hg were so low that they could not be detected by the adopted method.

### Agronomic evaluation of substrates - experiment 1

In some cases significant "GRH  $\times$  ADR" interactions were detected and are presented above. Where interaction resulted as not significant the main factor will be discussed.

Tomato. Plant leaf number was only affected by ADR addition and resulted as 7.8% higher in ADR-fertilized substrates (Tab. 4). Stem diameter of plants grown in 0- control was similar to those grown in 0+, 33- and 67-, while 100- showed lower values. ADR addition improved values in substrates with GRH (Fig 1). Hypocotyl length decreased with increasing of GRH rate while ADR addition increased values up to those of 0- (Fig. 2). Tomato transplants height showed similar behavior to that of hypocotyl length, with the difference that plants grown in 33+ and 67+ resulted as taller than the control (Fig. 3). Interaction "GRH x ADR" in fresh weight (Fig. 4) showed that this parameter was similar in substrates without ADR up to 67% GRH. 100- presented plants 35.6% lighter than the control. Addition of ADR did not affect weights in 0+ compared to the control, while this parameter improved in 33+, 67+ and 100+. Compared to transplants grown in the 0% GRH substrates, those grown with 33% GRH had a higher dry weight (+25.3%) (Tab. 4). A further increase of GRH led to a reduction in dry weight and, compared with the higher value (in 33% GRH) only those with 100% GRH were significantly lower. Plants grown in ADR-fertilized substrates presented 70.7% higher dry weight than those grown in ADRfree substrates. Dry matter content was only affected by ADR and resulted as 7% higher in plants grown in ADR-fertilized substrates (Tab. 4).

Salvia officinalis. Leaf number, stem diameter and dry matter content did not result as being significant for any studied factor or interaction (Tab. 4). Interaction "GRH x ADR" did not affect any parameter (Tab. 4). Hypocotyl length, plant height and dry weight were only affected by ADR addition (Tab. 4): plants grown in ADR-fertilized substrates presented 13.1, 7.0 and 22.6% higher values respectively than those in ADR-free substrates (Tab. 4). Plant fresh weight was only higher with 33% GRH, while no differences were observed with other GRH quantities. ADR addition generally improved plant fresh weight (+13.2%) (Tab. 4). Salvia splendens. The interaction "GRH x ADR" significantly affected all parameters. Leaf number (Fig. 5) declined with 67% or more GRH in the substrate in ADR-free substrates while ADR addition increased this parameter. No differences were observed in substrates with 0 or 33% GRH, which presented higher values. Stem diameter (Fig. 6), hypocotyl length (Fig, 7) and dry weight (Fig, 8) have similar behavior to leaf number but in these cases only 0+ presented values not different to the control. In particular hypocotyl length of plants grown on substrates with 0% GRH was greater than those with 33% GRH (on average 24.5%) and no differences were observed adding ADR. Further increasing of GRH in substrates decreased values only in substrates without ADR (on average -21.1%). Taller plants (Fig. 9) were observed in 33-, which were different from 0+, 67-, 100- and 100+. However control plant height did not differ from all the studied thesis. Aerial part fresh weight (Fig. 10) had similar behavior to stem diameter and hypocotyl length, with the difference that 33+ as well as 0+ presented values similar to the control. Control dry matter values (Fig. 11) were similar to those of 0+, 33-, 33+ and 100+. Lower values were observed for plants grown in the other substrates.

*Begonia semperflorens.* Except for dry weight all the vegetative parameters considered in Begonia resulted as being significantly affected by the interaction GRH x ADR (Tab. 4). Plants sown in 67- and 100- did not germinate, giving no results for any parameter considered. For leaf number (Fig. 12), height (Fig. 13) and fresh weight (Fig. 14) a remarkable decreasing of values was observed with the increasing GRH percentage in the substrates. Compared to 0-, addition of ADR decreased values of 0+ plants, while parameters slowly improved when GRH was present in the growing medium. However plants grown in 0% GRH presented 2.5, 3.2 and 12 times lower values than those grown in 33% GRH, 67+ and 100+ respectively. Dry weight was affected only by GRH content. Plants grown in 0 GRH% presented 13.6 times higher values compared to substrates with GRH in the mix (Tab. 6). Dry matter content was affected by substrates 67- and 100-. There were no significant differences in dry matter of plants grown in the other substrates.

In this experiment growth was reduced more significantly for *S. splendens* and *B. semperflorens* than for tomato and *S. officinalis*, with increasing rate of GRH in the substrates. Zanin et al. (2011) observed a similar behavior for tomato and chicory transplants that resulted as not or less sensitive respectively than pepper to increasing rate

of GRH. Kashyap and Panda (2003) outlined that water stress is the main limiting factor for potato and pepper growth and Oh et al. (2007) observed a reduction of some biometric parameters in kalanchoe linked to physical properties of different substrates when water supply was reduced. Hence according to Zanin et al. (2011) plants growth depletion is probably due to the inferior physical properties (e.g. WHC and EAW) respect of the chemical and nutritional change observed in the substrates with the addition of GRH (e.g. reduction of NO3-N and Ca, increase of K and Mn).

The addition of ADR actually worsens the physical properties, but the much higher nutrient level probably allows the better transplant growth. This was also seen by some authors who added other decomposed biomasses (composted agro-wastes, manure or sewage sludges) to growing media and observed an improvement of biometric parameters of ornamental plants and tomato seedlings thanks to higher nutrient level content (Atiyeh et al., 2001; Garcia-Gomez, 2002; Ostos et al., 2008; Herrera et al., 2008).

### Agronomic evaluation of substrates - experiment 2

*Tomato*. In this experiment 3 to 6 more days were needed to achieve marketable size (Tab. 5). Leaf number was not affected by the main factor or the interaction "GRH x ADR" (Tab. 6). Stem diameter, hypocotyl length, height and fresh weight were affected by the interaction "GRH x ADR" (Tab. 6). Stem diameter (Fig. 15) and fresh weight (Fig. 16) with increasing GRH% were reduced only with 100% GRH. ADR addition improved these parameters in substrates with GRH. However compared to 0-, only 33+ and 67+ presented higher values and 100- lower. Hypocotyl lengths of 0- were comparable to all other substrates, with the exception of 67- and 100- that presented lower values (Fig. 17). Similar behavior was observed for plant height (Fig. 18), but only plants grown in 100- resulted as being smaller than the control. No differences were observed on dry weight (Fig. 19) and dry matter (Fig. 20) of plants grown in the control substrate compared to the others even though ADR addition to 67% GRH promoted (+41.9%) plant dry weight (Fig. 19).

*Salvia officinalis.* The cultivation cycle of *S. officinalis* was 3 days longer for plants grown in 67+ and 100+ and 4 days longer for those in 67- and 100- (Tab. 5). Fresh and dry weight were 11.3 and 18.1% higher respectively in plants grown in ADR-fertilized substrates (Tab. 6). No other parameter resulted as being affected by the studied factors (Tab. 6).

Salvia splendens. Five supplementary days were required by plants grown in 67+ and 100+ while 15 days were needed with 67- and 100- (Tab. 5). Leaf number, fresh weight and dry weight did not show any differences between substrates (Tab. 6). The interaction "GRH x ADR" affected only plant height, where a few differences were observed between substrates. In particular shorter plants were obtained in substrate 67- while those grown in 0- were similar to the others (Fig. 21). Stem diameter resulted as lower in substrates with GRH while ADR addition generally increased values by 5.3% (Tab. 6). Hypocotyl length resulted as higher in substrates with 0% GRH and was reduced by 23.5 and 33.0% respectively in substrates with 33 and 100% GRH (Tab. 6). Dry matter percentage was reduced by 19.5% with ADR addition (Tab. 6).

*Begonia semperflorens*. After 50 days from first measurement all plants were measured and the trial was stopped even though plants were not marketable (Tab. 5). The interaction "GRH x ADR" affected leaf number, plant height, fresh weight and dry matter content (Tab. 6). Compared to 0-, in 50 days differences observed for leaf number (Fig. 22) and fresh weight of aerial part (Fig. 23) were partially reduced but all values were lower than those of the peat control. Plant height (Fig. 24) of the control (0-) was similar to those grown in substrates 0+ and 33+, but again lower values were obtained using other substrates. Dry weight was strongly reduced adding GRH to substrates, while ADR addition gave 1.56 times heavier plants (Tab. 6). Dry matter of plants grown in the peat control (Fig. 25) was not different from the other substrates, with the exception of 67- and 100- that produced no plants.

In this experiment tomato and *S. officinalis* plants gave in general comparable values to the first that reached marketable size. Generally GRH did not affect plant quality but only the timing of the cultivation cycle. *S. splendens* resulted as being more sensitive to increasing GRH% and this was observed also with a longer growing cycle in substrates with higher GRH content. *B. semperflorens* growth was heavily affected by GRH content. The longer cultivation cycles probably are due to a lack of water caused by low WHC and EAW that reduced the growth especially in *S. splendens* and *B. semperflorens*.

As seen in experiment 1, ADR generally supported the growth of plants probably because of the higher nutrient elements content.

## Conclusions

Physical properties resulted as being negatively affected by increasing GRH content in substrates. It decreased TPS increasing AFP. As a consequence WHC, AW, EAW and WBC were reduced. This means that a change in water management is required, with frequent but lower water volumes. ADR addition improved WHC, AW, EAW only in substrates with 0 and 33% GRH.

Under chemical aspects, GRH decreased CEC to more suitable levels and increased DM, OM and OC. It also increased P and K availability in the water extract but reduced the concentration of the other nutrient elements (N, Ca, Mg) causing a possible nutritional imbalance. This problem was partially overcome with ADR addition because it increased all tested nutrient elements.

In experiment 1, for tomato seedling production a partial substitution of peat with GRH is possible in substrates (33 or 67%) if ADR are added to the mix. A total substitution of peat with GRH is possible to produce *S. officinalis* seedlings. In this case ADR addition did not affect seedling growth. For production of *S. splendens* and *B. semperflorens* no peat substitution by GRH is possible as they resulted as being more sensitive to worsening of physical properties with a GRH increase in the substrate. In general, when the rate of GRH was higher than 33%, seedlings did not reach marketable size at the same time as the peat control. The addition of ADR for these ornamental productions improved plant parameters but without reaching the same level as substrates without GRH.

In experiment 2, tomato, *S. officinalis* and *S. splendens* plants showed few or no differences between plants that reached marketable size first even if *S. splendens* needed a longer time with respect to the other species to reach marketable size. Unfortunately *B. sempeflorens* never reached acceptable standard when it was grown in substrates with GRH, even if ADR addition permitted a minimal production.

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# **Tables**

Table 1. Effects of interaction "rate of GRH (A)  $\times$  ADR treatment (B)" on bulk density (g cm<sup>-3</sup>), total pore space (TPS), air-filled porosity (AFP), water holding capacity (WHC), available water (AW), easily available water (EAW), water buffer capacity (WBC) and unavailable water (UW) of the substrates (values indicate % by volume).

	Substrate									Significance <sup>^</sup>		
	0-	33-	67-	100-	0+	33+	67+	100+	А	В	A×B	
BD (g cm <sup>-3</sup> )	0.264 f	0.267 f	0.267 f	0.299 e	0.377 a	0.352 b	0.340 c	0.326 d	***	***	***	
TPS (%)	90.4 a	70.8 bc	67.4 c	76.1 b	87.7 a	70.4 bc	67.1 c	81.2 b	***	n.s.	n.s.	
AFP (%)	45.0 b	23.8 c	33.9 b	48.1 ab	30.9 bc	26.4 c	35.9 b	53.0 a	***	**	**	
WHC (%)	45.4 b	43.9 b	33.5 bc	29.3 c	56.9 a	47.1 b	31.2 c	27.0 c	***	**	**	
AW (%)	20.4 b	18.5 bc	9.5 d	7.7 de	23.2 a	17.3 c	6.0 e	6.3 e	***	n.s.	***	
EAW (%)	16.2 a	15.7 a	7.6 b	5.5 b	18.9 a	14.9 a	4.4 b	4.5 b	***	n.s.	*	
WBC (%)	4.06 a	2.75 b	1.82 b	2.24 b	4.22 a	2.35 b	1.64 b	1.84 b	***	n.s.	n.s.	
UW (%)	25.0 bc	28.4 b	24.0 cd	21.1 d	33.7 a	27.7 bc	25.2 bc	17.2 e	***	n.s.	***	

Values with the same letter are not different according to Tukey's HDR test ( $P \le 0.05$ ).

 $\uparrow$ : \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant

	Substrate										
Parameter	0-	33-	67-	100-	0+	33+	67+	100+	Sign.^		
рН	5.69 f	6.06 d	6.04 d	6.27 c	7.00 a	6.51 b	6.24 c	5.80 e	***		
CEC (meq 100g <sup>-1</sup> )	146 a	85.1 c	50.3 e	32.7 f	116 b	86.2 c	61.2 d	40.6.ef	***		
$EC (mS cm^{-1})$	0.32 d	0.24 e	0.34 d	0.48ab	0.50 a	0.32 d	0.41 c	0.50 a	***		
TKN (%)	0.81 d	0.57 e	0.46ef	0.40 f	1.97 a	1.43 b	1.04 c	1.09 c	***		
C/N ratio	53	81	104	119	20	31	44	42	-		
$P (mg L^{-1})$	1.71 c	4.53 c	11.0 b	16.6	16.6	18.3 a	19.5 a	17.0	***		
$K (mg L^{-1})$	5.7 e	36.9	85.0	116 a	50.9	79.8	99.0	83.0	***		
Ca (mg $L^{-1}$ )	8.86	8.74	6.5 de	2.87 e	12.4	15.8	18.7 a	14.7	**		
$Mn (mg L^{-1})$	0.028	0.123	0.296	0.814	0.035	0.114	0.233	0.407	***		
Cr (mg kg <sup>-1</sup> )	0.65 c	0.35 c	0.12 c	0.10 c	4.85 a	2.82 b	2.34 b	2.55 b	**		
Pb (mg kg <sup>-1</sup> )	3.02 a	1.32 d	0.68 e	0.30 f	2.63 b	1.82 c	1.07 d	0.68 e	***		

Table 2. Effects of interaction "rate of GRH × ADR treatment" on some chemical features of the substrates.

Values with the same letter are not different according to Tukey's HDR test ( $P \le 0.05$ ).

^ significance of interaction (both rate of GRH and ADR treatment were significant at  $P \le 0.001$ ): \*\*\* and \*\* = significant at  $P \le 0.001$  and 0.01 respectively.

Table 3. Effects of rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on some chemical features of the substrates (OM is organic matter and OC organic carbon).

	GRH (%)				AĽ	DR	Sign. ^		
Parameter	0	33	67	100	-	+	GRH	ADR	
OM (%)	71.4 c	77.4 b	80.8 a	81.2 a	79.8 a	75.6 b	***	***	
OC (%)	41.4 c	44.9 b	46.8 a	47.1 a	46.3 a	43.9 b	***	***	
NO <sub>3</sub> -N (mg L <sup>-1</sup> )	30.4 a	13.2 b	3.2 c	1.5 c	12.5	11.6	***	n.s.	
						n.s.			
$Mg (mg L^{-1})$	7.91 a	8.90 a	7.99 a	5.65 b	7.03 b	8.19 a	***	**	
Al (mg kg <sup>-1</sup> )	1019 a	546 b	345 c	246 c	284 b	795 a	***	***	
Cu (mg kg <sup>-1</sup> )	51.9 a	27.3 b	23.6 b	27.4 b	11.2 b	53.9 a	***	***	
Ni (mg kg <sup>-1</sup> )	1.97 a	1.25 b	0.82 bc	0.64 c	0.58 b	1.76 a	***	***	
Zn (mg kg <sup>-1</sup> )	34.9 a	26.2 b	21.6 bc	20.1 c	16.4 b	35.0 a	***	***	

Values with the same letter are not different according to Tukey's HDR test ( $P \le 0.05$ ).

^: \*\*\* and \*\* = significant at  $P \le 0.001$  and 0.01 respectively. n.s. = not significant

Table 4. Experiment 1: effects of rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) in the substrates on transplant growth.

		GRH (%)				А	DR		Significance <sup>^</sup>		
Species	Parameter	0	33	67	100	-	+	GRH	ADR	GRH×ADR	
Tomato	Leaf number	3.64	3.68	3.60	3.45	3.46 b	3.73 a	n.s.	***	n.s.	
	Stem diameter (mm)	1.98 bc	2.18 a	2.11 ab	1.93 c	1.89 b	2.21 a	**	***	***	
	Hypocotyl length (cm)	4.69	4.62	4.56	4.09	4.20 b	4.79 a	n.s.	***	***	
	Height (cm)	13.0 ab	14.8 a	13.9 ab	12.1 b	12.1 b	14.8 a	*	***	***	
	Fresh weight (mg)	613 b	800 a	705 ab	584 b	523 b	828 a	**	***	***	
	Dry weight (mg)	78.0 ab	97.8 a	83.3 ab	73.3 b	61.5 b	105 a	*	***	n.s.	
	Dry matter (%)	12.6	12.2	11.7	12.2	11.7 b	12.6 a	n.s.	*	n.s.	
S. officinalis	Leaf number	10.85	10.63	10.00	10.03	10.25 b	10.50 a	n.s.	n.s.	n.s.	
	Stem diameter (mm)	1.86	1.98	1.90	1.74	1.84	1.90	n.s.	n.s	n.s.	
	Hypocotyl length (cm)	4.44	3.91	4.84	5.05	4.28 b	4.84 a	n.s.	**	n.s.	
	Height (cm)	8.50	8.96	8.95	8.70	8.48 b	9.07 a	n.s.	**	n.s	
	Fresh weight (mg)	564 b	656 a	584 b	569 b	554 b	633 a	*	**	n.s.	
	Dry weight (mg)	106	119	102	99.3	95.8 b	118 a	n.s.	**	n.s.	
	Dry matter (%)	19.26	18.84	18.15	18.00	17.99	19.14	n.s.	n.s.	n.s.	
S. splendens	Leaf number	8.53 a	7.99 a	6.81 b	6.39 b	6.97 b	7.89 a	***	***	***	
-	Stem diameter (mm)	1.50 a	1.31 b	1.17 c	1.08 c	1.21 b	1.32 a	***	***	**	
	Hypocotyl length (cm)	4.43 a	3.39 b	2.75 bc	2.61 c	3.03 b	3.56 a	***	***	***	
	Height (cm)	6.89 ab	7.95 a	6.90 ab	6.35 b	7.07	6.97	*	n.s.	**	
	Fresh weight (mg)	293 a	235 a	161 b	134 b	172 b	239 a	***	***	***	
	Dry weight (mg)	32.8 a	18.5 b	8.4 bc	7.4 c	14.0 b	19.5 a	***	*	*	
	Dry matter (%)	11.2 a	7.44 ab	4.18 b	5.19 b	6.27 b	7.73 a	**	**	*	
B. semperflorens	Leaf number	7.40 a	2.93 b	1.55 c	1.38 c	2.64 b	3.98 a	***	***	***	
	Height (cm)	1.03 a	0.32 b	0.19 c	0.14 c	0.34 b	0.50 a	***	***	***	
	Fresh weight (mg)	404 a	37 b	18 b	12 b	123	112	***	n.s.	***	
	Dry weight (mg)	23.2 a	2.89 b	1.30 b	0.86 b	6.40	7.73	***	n.s.	n.s.	
	Dry matter (%)	8.01 a	9.59 a	5.69 b	4.37 b	3.83 b	10.0 a	***	***	***	

Values with the same letter are not different according to Tukey's HDS test ( $P \le 0.05$ ).

 $\uparrow$ : \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant

Species	0-	33-	67-	100-	0+	33+	67+	100+
Tomato	3	3	3	6	0	0	3	5
S. officinalis	0	0	4	4	0	0	3	3
S. splendens	0	0	15	15	0	0	5	5
B. semperflorens	0	50	n.d.	n.d.	50	50	50	50

Table 5. Experiment 2: additional days needed to reach marketable size of seedlings.

n.d.= not detected

Table 6. Experiment 2: effects of rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) in the substrates on transplant growth.

		GRH (%)			A	ADR			Significance <sup>^</sup>		
Species	Parameter	0	33	67	100	-	+	GRH	ADR	GRH×ADR	
Tomato	Leaf number	3.75	3.83	3.84	3.92	3.80	3.87	n.s.	n.s.	n.s.	
	Stem diameter (mm)	1.98 bc	2.18 a	2.11 ab	1.93 c	1.89 b	2.21 a	**	***	**	
	Hypocotyl height (cm)	46.94	46.18	45.64	40.93	41.95 b	47.89 a	n.s.	***	**	
	Height (cm)	12.98 ab	14.83 a	13.94 ab	12.07 b	12.07 b	14.84 a	*	***	**	
	Fresh weight (mg)	613 b	800 a	705 ab	584 b	523 b	828 a	**	***	*	
	Dry weight (mg)	78.00 ab	97.75 a	83.25 ab	73.25 b	62.50 b	104.62 a	*	***	n.s.	
	Dry matter (%)	12.58	12.18	11.66	12.21	11.75 b	12.57 a	n.s.	*	n.s.	
S. officinalis	Leaf number	10.85	10.63	10.79	10.68	10.69	10.78	n.s.	n.s.	n.s.	
	Stem diameter (mm)	1.86	1.98	1.95	1.82	1.87	1.93	n.s.	n.s.	n.s.	
	Hypocotyl height (cm)	0.44	0.39	0.48	0.52	0,43	0.49	n.s.	n.s.	n.s.	
	Height (cm)	8.50	8.96	9.73	9.49	8.98	9.36	n.s.	n.s.	n.s.	
	Fresh weight (mg)	564	656	651	637	594 b	661 a	n.s.	*	n.s.	
	Dry weight (mg)	106	119	118	115	105 b	124 a	n.s.	**	n.s.	
	Dry matter (%)	19.26	18.84	18.58	18.49	18.32	19.27	n.s.	n.s.	n.s.	
S. splendens	Leaf number	8.53	7.99	8.15	7.98	8.09	8.23	n.s.	n.s.	n.s.	
	Stem diameter (mm)	1.50 a	1.31 b	1.34 b	1.24 b	1.31 b	1.38 a	**	*	n.s.	
	Hypocotyl height (mm)	4.43 a	3.39 b	3.78 ab	3.33 b	3.66	3.80	**	n.s.	n.s.	
	Height (cm)	689 ab	795 a	579 b	651 b	673	684	**	n.s.	*	
	Fresh weight (mg)	293	235	272	224	245	267	n.s.	n.s.	n.s.	
	Dry weight (mg)	32.75	18.50	29.54	23.92	26.50	26.10	n.s.	n.s.	n.s.	
	Dry matter (%)	11.17	7.44	11.45	10.43	11.22 a	9.03 b	n.s.	*	n.s.	
B. semperflorens	Leaf number	7.40 a	5.20 b	2.55 c	1.95 c	3.08 b	5.47 a	***	***	***	
	Height (cm)	1.03 a	0.75 a	0.38 b	0.32 b	0.41 b	0.84 a	***	***	**	
	Fresh weight (mg)	0.40 a	0.18 b	0.08 b	0.05 b	0.14 b	0.21 a	***	*	**	
	Dry weight (mg)	23.21 a	7.35 b	3.73 b	2.68 b	7.20 b	11.29 a	***	*	n.s.	
	Dry matter (%)	8.01 a	5.82 ab	5.34 ab	3.60 b	3.34 b	8.04 a	*	***	**	

Values with the same letter are not different according to Tukey's HDS test ( $P \le 0.05$ ).

 $\uparrow$ : \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant

# **Figures**



Fig. 1. Experiment 1. Effect of interaction "GRH × ADR" on tomato stem diameter. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 2. Experiment 1. Effect of interaction "GRH × ADR" on tomato hypocotyl length. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 3. Experiment 1. Effect of interaction "GRH × ADR" on tomato height. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 4. Experiment 1. Effect of interaction "GRH × ADR" on tomato fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.


Fig. 5. Experiment 1. Effect of interaction "GRH × ADR" on S. splendens leaf number. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 6. Experiment 1. Effect of interaction "GRH × ADR" on S. splendens stem diameter (mm). (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 7. Experiment 1. Effect of interaction "GRH × ADR" on S. splendens hypocotyl length. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 8. Experiment 1. Effect of interaction "GRH × ADR" on S. splendens dry weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 9. Experiment 1. Effect of interaction "GRH × ADR" on S. splendens plant height. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 10. Experiment 1. Effect of interaction "GRH × ADR" on S. splendens fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 11. Experiment 1. Effect of interaction "GRH × ADR" on S. splendens dry matter percentage. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 12. Experiment 1. Effect of interaction "GRH × ADR" on *B. semperflorens* leaf number. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 13. Experiment 1. Effect of interaction "GRH × ADR" on *B. semperflorens* height. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 14. Experiment 1. Effect of interaction "GRH × ADR" on *B. semperflorens* fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 15. Experiment 2. Effect of interaction "GRH × ADR" on tomato stem diameter. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 16. Experiment 2. Effect of interaction "GRH × ADR" on tomato fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 17. Experiment 2. Effect of interaction "GRH × ADR" on tomato hypocotyl length. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 18. Experiment 2. Effect of interaction "GRH × ADR" on tomato height. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 19. Experiment 2. Effect of interaction "GRH × ADR" on tomato dry weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 20. Experiment 2. Effect of interaction "GRH × ADR" on tomato dry matter percentage. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 21. Experiment 2. Effect of interaction "GRH × ADR" on *S. splendens* height (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 22. Experiment 2. Effect of interaction "GRH × ADR" on *B. semperflorens* leaf number. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 23. Experiment 2. Effect of interaction "GRH × ADR" on *B. semperflorens* fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 24. Experiment 2. Effect of interaction "GRH × ADR" on *B. semperflorens* height. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 25. Experiment 2. Effect of interaction "GRH × ADR" on *B. semperflorens* dry matter percentage. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.

**Chapter III** 

Rice hull-based substrates amended with anaerobic digested residues for tomato transplant production

# Abstract

Some agricultural byproducts may be suitable as peat alternatives in root substrates, such as rice hulls, or as fertilizers, such as anaerobic digested residues (ADR). In this study four substrates were prepared with increasing rates (0, 33, 67 and 100%) of 2 mm ground rice hulls (GRH) as substitution for peat. These mixes were, or not, amended with 20% (by volume) of ADR. Substrates were physically and chemically analyzed and a cultivation trial was conducted in order to evaluate 'Jack' tomato seedlings growth, under three irrigation regimes (IR). Water distribution was 225 ml  $d^{-1}$ , 450 ml  $d^{-1}$  and 900 ml  $2d^{-1}$  per tray. Total pore space (TPS) was highest in 0% GRH substrates while air filled porosity (AFP) was highest in 100% GRH substrates. Water holding capacity and easily available water decreased along with the increase in GRH and differences between substrates were increased by ADR. ADR reduced TPS and AFP, but not in all mixes. Electrical conductivity and nutrient contents were higher when ADR was added. In the cultivation trial, plant height, leaf number and area, fresh and dry weight were negatively affected by increasing GRH rates, while fertilization with ADR improved plant growth. Among the different IR, 450 ml d<sup>-1</sup> guaranteed better biomass performances compared to the other treatments.

## Introduction

Among all the materials evaluated to substitute peat in root substrates, rice hulls are interesting because, being a byproduct of the rice milling industry, they have a relatively low cost and are easily available in large volumes (Del Amor, 2009). Fresh rice hulls have high air porosity and low water holding capacity (WHC) (Bunt, 1988; Hanan, 1998). Because of this, the more reliable use of fresh and parboiled rice hulls has so far been just as substitute for mineral components, such as perlite or vermiculite, which provide aeration in peat-based substrates (Evans and Gachukia, 2004; Evans and Gachukia, 2007). Differently processed rice hulls, such as carbonized and composted, have higher WHC (Lovelace and Kuczmarski, 1994; Martins-Medeiros et al., 2001). However, the wide use of these products is limited by the environmentally unfriendly process that carbonized rice hulls have to undergo (the carbonization itself), and the lack of consistency of the standard method of production, and thus constancy of characteristics, of the composted hulls. Lately

several researches have pointed out that the physical properties of ground fresh rice hulls (GRH) are much closer to those of peat (Sambo et al., 2008; Buck and Evans, 2010). Studies on vegetable and flower transplants production reported that the progressive substitution of peat with GRH often gradually reduced growth (Zanin et al., 2011; Bassan et al., in press).

Sewage sludge, the organic fraction of municipal wastes and agricultural byproducts can be exploited to produce energy through anaerobic digestion. Final residues of this process generally have low C/N ratio and high nutrients level; these materials are hence interesting for agricultural uses as fertilizers or amendants (Marchaim et al., 1991; Shi et al., 2002). Recently anaerobic digestion biosolids derived from cattle manure were used in substrates to grow ornamentals with good results (Compton and Zauche, 2006a; Compton and Zauche, 2006b). However little is known about the characteristics of substrates containing anaerobic digested residues (ADR), or plant response.

The aim of this study was to evaluate the chemical, physical and agronomical properties of some substrates containing GRH and ADR in different rates as component of substrates for transplant production. In the agronomical evaluation, different irrigation regimes (IR) were tested in order to better meet different physical properties of substrates.

## Material and Methods

#### Substrates preparation

Four substrates were prepared by combining fresh rice hulls, ground at 2 mm (GRH), with a commercial peat-based substrate commonly used for transplant production (hereafter named peat). The relative ratios were 0, 33, 67 and 100% by volume of GRH, with the remainder being peat. These substrates were then amended with ADR deriving from fruit distillery wastes. ADR were considered as fertilizer. ADR-free substrates were named 0-, 33-, 67- and 100- and those containing ADR 0+, 33+, 67+ and 100+, respectively.

## Physical and chemical properties of substrates

Four substrates were prepared by combining fresh rice hulls, ground at 2 mm (GRH), with a commercial peat-based substrate commonly used for transplant production (hereafter named peat). The relative ratios were 0, 33, 67 and 100% by volume of GRH, with the remainder being peat. These substrates were then amended with ADR deriving from fruit distillery wastes. ADR were considered as fertilizer. ADR-free substrates were named 0-, 33-, 67- and 100- and those containing ADR 0+, 33+, 67+ and 100+, respectively.

Substrates were characterized from the physical and chemical standpoint on five samples each. Physical-hydraulic properties of substrates were evaluated by means of Tempe cell 1400 (Soilmoisture Equipment Corporation, Santa Barbara, USA) imposing pressures of -1, -2, -5 and -10 kPa. Total pore space (TPS), air-filled porosity (AFP), water holding capacity (WHC), easily available water (EAW) and water buffer capacity (WBC) and non available water (NAW) were determined according to Bunt (1988) and De Boodt and Verdonck (1972). For other physical (bulk density, BD) and chemical characterization the methods described in chapter 2.

#### Agronomic evaluation of substrates

The 8 substrates were also agronomically evaluated for 'Jack' tomato transplant production. The experiment was set in a glass greenhouse with openings in the roof and

side walls. Seedlings were grown in plastic trays with 300 cells (135 mm3 volume each). Trays were hand-sown on October 26, covered with a vermiculite layer, watered and moved to the greenhouse. After seed germination (8 days after sowing) the following irrigation regimes (IR) were applied: 225 ml d-1, 450 ml d-1 and 900 ml 2d-1 of water per tray. Starting from 14 days after sowing trays were fertigated every 5 days (4 times in total) using 0.8 g L-1 of a 13N-2.2P-16.7K plus microelements hydro-soluble fertilizer. The experiment was concluded 38 days after sowing at which time seedling height, number of true leaves, fresh and dry weight and dry matter of aerial parts of plants, and leaf area of 10 plants for each treatment and block were evaluated.

## Data statistical analysis

Data were analyzed by analysis of variance (ANOVA) and means separated according to Tukey's HSD test ( $P \le 0.05$ ). Data on physical-chemical characterization of substrates were analyzed as a factorial experiment in a two-way completely randomized design while for the cultivation trial a three-way ANOVA design with four replications was adopted. When necessary, values expressed as percentage were angular transformed before analysis.

# **Results and Discussion**

#### Physical properties of substrates

Relative rate of GRH in the substrates interacted with presence of ADR on affecting BD (Table 1). In the absence of ADR, differences were observed among substrates containing 33 and 67% GRH compared to 100% GRH. Addition of ADR increased BD but a progressive reduction in values was observed with increasing rates of GRH. The different trend observed with ADR-containing compared to the ADR-free substrates is probably due to different settling and packing of particles with different shape and size, which are less predictable when three components are mixed in a substrate compared to two (Buck and Evans, 2010). Furthermore, values are consistent with those we obtained in a previous experiment (Bassan et al., in press). All values, however, are slightly higher than the recommended level for transplants (Aendekerk et al., 2000).

TPS and AFP varied, along with the increase of GRH in the substrate, with a boatshaped pattern (Table 1). TPS values in 0% GRH substrates were similar (on average 88.7%), in both ADR-containing and ADR-free substrates. Values were lowest in the other substrates (on average 68.9%), with the exception 100- substrate in which the value was intermediate (79.6%). AFP was higher in the 0- than the 0+ substrate (45.0 vs. 30.9%), then slightly decreased in 33% GRH substrates. Further increase of GRH led to a significant increase of AFP without any difference between ADR-free and -containing substrates (Table 1). In 0% GRH substrates WHC was higher when ADR was added (56.3 vs. 45.1%); increasing rates of GRH then reduced WHC but to a minor extent in ADR-free substrates. EAW and WBC were affected only by rate of GRH. Values were highest in 0% GRH substrates (on average, 15.6 and 4.3% respectively) and lowest in 100% GRH (on average 4.3 and 1.42%, respectively) (Table 1). While NAW in ADR-free substrates was not affected by increasing rates of GRH, in ADR-containing substrates these values decreased along with the increase of GRH rate. Data are consistent with those of other authors (Sambo et al., 2008; Bassan et al., in press) who also used GRH.

According to several authors (Boertje, 1984; Jenkins and Jarrell, 1989) the optimal range for TPS in substrates is 60-85%. Bunt (1988) and Jenkins and Jarrell (1989) recommended an AFP of 10- 20%. The optimal WHC has been reported to be about 45%

(Boertje, 1984) and the EAW 20-30% (De Boodt and Verdonck, 1972). Hence, all substrates had reasonably good TPS and high AFP. Substrates with 67 or 100% GRH had low WHC and all had low EAW values.

### **Chemical properties of substrates**

The analysis of variance conducted on chemical characteristics revealed an interaction effect between the two factors in many cases, the results of which are reported in table 2. When the interaction was not significant data are reported in table 3.

In ADR-free substrates, pH was lower in 0% GRH than those containing any rate of GRH (from 5.37 to 5.78, on average) (Table 2). In general, in substrates containing ADR, pH values were higher, particularly in those containing low rates of GRH (6.1 and 6.33 in 0+ and 33+ substrates). All the substrates presented pH values within optimal values (Bunt, 1988). Electrical conductivity (EC) values were higher in ADR-containing than in ADR-free substrates (on average 0.36 vs. 0.75 mS cm-1). Furthermore, while no differences were observed in ADR-free substrates, when ADR was added EC was higher in the 0% GRH substrate than in GRH-containing substrates (1.1 vs. 0.6 mS cm-1) (Table 2). As advisable EC levels are lower than 0.5 mS cm-1 (Pozzi and Valagussa, 2009), ADR-containing substrates had relatively high values, especially in 0% GRH substrate. With increasing GRH rate in ADR-free substrates a decrease in cation exchange capacity (CEC) was observed (from 141 to 29 cmol kg-1 in 0 and 100% GRH substrates, respectively). A reduction in CEC was also found in substrates containing ADR, but the variation was smaller (from 112 to 37 cmol kg-1) (Table 2). CEC value of peat in this study was lower than values found by Benito et al. (2006) but higher than those of Abad et al. (2002).

Values of total Kjeldahl nitrogen (TKN) were, in general, higher in ADR-containing compared to ADR-free substrates (on average 0.46 vs. 1.51 %). Furthermore, with increasing GRH rate in the substrate TKN decreased (from 0 to 100% GRH) by 64.5% and 48.7% in ADR-free and ADR-containing substrates respectively (Table 2). C/N ratio was much higher in ADR-free than in ADR-containing substrates and values increased with the increasing of GRH rate (Table 2). Hence, the substrates in which ADR were added and those with a low rate of GRH are less prone to nitrogen immobilization during plant growth. Even if a statistical analysis for this parameter was not possible because of the independence of samples, comparable values were observed in a similar study (Bassan et

al., in press). Concentration of NO<sub>3</sub>-N (Table 2) of substrates containing 0% GRH was higher when ADR were added to the substrates (48.3 vs. 21.5 mg  $L^{-1}$ ), the increase of GRH rate strongly reduced NO<sub>3</sub>-N concentration and no difference was observed between ADR-free and ADR-containing substrates. According to Pozzi and Valagussa (2009), normal values range between 11 and 23 mg  $L^{-1}$ . Hence, in 0% GRH substrates values are high while, in those with 67 or 100% GRH, values are low.

In ADR-free substrates, the increase of GRH rate from 0 to 100% resulted in a more than four and eight times increase of P and K concentrations, respectively. Values in ADRcontaining substrates were higher and increased slightly (P) or not at all (K) when GRH rate was raised (Table 2). Data are consistent with those obtained in a previous experiment (Bassan et al., in press). According to Pozzi and Valagussa (2009), normal ranges for P and K are 6.1-8.3 and 4-14 mg L<sup>-1</sup>. Thus ADR-free substrates with low GRH rates tend to be low in P, while substrates containing ADR and high GRH rates tend to have excessive P. The substrates containing only peat had a concentration of K within the advisable range while all the others had very high concentrations. The high values of K of substrates containing rice hulls is already known, particularly if ground (Zanin et al., 2011). Concentrations of Ca and Mg had a similar pattern (Table 2). In ADR-free substrates concentrations were highest in 0% GRH substrates (on average 34.8 and 9.2 mg L<sup>-1</sup>, for P and K respectively). Increasing rates of GRH reduced concentrations of both nutrients with no difference between treatments (on average 9.7 and 2.6 mg  $L^{-1}$  respectively). Normal values of Ca and Mg in the water extract are 10-19 and 6-10 mg L<sup>-1</sup> (Pozzi and Valagussa, 2009). Only 33% GRH and 67% GRH with ADR substrates for Ca, and both 0% GRH substrates for Mg are within normal values.

As GRH in the substrate increased from 0 to 100%, OM and OC rose from 71.2 to 80.7% and from 41.3 to 46.8%, respectively. The addition of ADR to the substrates reduced MC and OC values by about 5% (Table 3). Mn concentration was affected only by GRH rate and increased up to 0.221 mg  $L^{-1}$  in 100% GRH substrates (Table 3). As already known, concentrations of Mn are generally very high in GRH (Evans and Gachukia, 2008; Zanin et al., 2011) and may be toxic for plants. In fact, 67 and 100% GRH substrates had higher values than recommended (Pozzi and Valagussa, 2009).

Concerning heavy metals, the concentrations of Cd and Hg in the dry matter were

not detected by the adopted method. Cu, Ni, Pb and Zn concentrations were very low compared to the Italian legal limit for substrates (Dlgs 75/10). Their concentrations decreased with addition of GRH, and ADR-containing substrates showed higher levels than the ADR-free ones (data not reported). Concentration of Cr showed the same trend and, in some cases, values exceeded legal limits. However, Italian law refers to hexavalent Cr, while in this study total Cr was evaluated (data not reported).

### Agronomic evaluation of substrates

The results of analysis of variance performed on the tomato transplant experiment are reported in table 4. Interaction effects were often observed and, in this chapter, only highly significant interactions ( $P \le 0.01$ ) are discussed.

Increasing GRH rate in the substrates, values of all parameters collected on tomato transplants were reduced, with the exception of dry matter percentage. Addition of ADR significantly improved plant growth. Tomato transplants presented higher height, leaf number and fresh weight when watered with 450 ml d-1, while not significant differences were observed between the other treatments (Table 4). Dry matter percentage was only affected by IR: plants watered with 225 ml d-1 had a higher dry matter (8.31%) content than those of the other treatments (on average, 6.78%; data not reported).

Interaction between GRH and ADR often affected transplants growth. Increasing GRH from 0 to 100%, in ADR-free substrates, plant height (Fig. 1), fresh weight (Fig. 2) and leaf area (Fig. 3) decreased by 42.1, 63.3 and 68.4%. Plants grown in ADR-containing substrates had higher values in 0% and 100% GRH substrates. As reported for tomato, pepper and red salvia transplants (Bassan et al., in press) growth was also reduced in this experiment with increasing GRH rate in the substrate. Plant growth may be partly affected by the lack of NO3-N, Ca and Mg, but particularly by the worse physical properties of GRH. The addition of ADR did not substantially changed physical properties but the higher nutritional level probably justifies the better transplant growth.

Plant height (Fig. 4) and fresh weight (Fig. 5) were strongly affected by the interaction between GRH and IR. Values were always higher with 450 ml d-1 than those obtained with the other IR especially at lower GRH rates.

Irrigation with 450 ml d<sup>-1</sup> is a normal practice with the peat-based substrate used in this experiment. The addition of ADR and GRH changed the substrate properties and in

particular the physical ones. Hence, differentiation in irrigation strategy is needed. None of the proposed regimes have met the different requirements of GRH- and ADR-containing substrates.

## Conclusions

Addition of GRH and ADR significantly modified both physical and chemical characteristics of substrates. GRH in general reduced WHC and increased AFP while ADR increased the nutritional level in the growing media. Tomato transplant growth was reduced by the increase of GRH rate in the substrates and in some cases supported, even if partially, by ADR. For this reason it is advisable not to exceed 33% of GRH in substrates. Lastly, independently of the substrate used, irrigation with 450 d-1 per tray was the best IR. Further researches are needed to fit irrigation regime to GRH substrates, for instance reducing the amount of irrigation water distributed and increasing irrigation frequency.

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# **Tables**

Table 1. Effects of interaction "GRH rate (A) × ADR treatment (B)" on bulk density (BD), total pore space (TPS), air-filled porosity (AFP), water holding capacity (WHC), easy available water (EAW) and water buffer capacity (WBC) of the substrates. Symbols – or + refer to addition or not of ADR.

		Sig	Significance <sup>^</sup>								
	0-	33-	67-	100-	0+	33+	67+	100+	А	В	A×B
BD	281 ef	275 f	272 f	291 e	365 a	354 b	334 c	326 d	***	**	**
TPS	90.1 a	70.7 c	67.5 c	79.6 b	87.3 a	70.4 c	67.6 c	68.3 c	***	**	**
AFP	45.0 a	28.0 bc	33.4 bc	50.3 a	30.9 bc	25.1 c	35.1 b	46.8 a	***	**	**
WHC	45.1 b	42.7 b	34.1 c	29.3 cd	56.3 a	45.3 b	32.5 c	21.5 d	***	n.s.	***
EAW	16.8 a	12.5 a	7.4 b	5.5 bc	14.4 a	13.8 a	4.5 c	3.2 c	***	n.s.	n.s.
WBC	4.2 a	1.8 bc	2.1 bc	1.7 bc	4.4 a	2.5 b	1.4 bc	1.0 c	***	n.s.	n.s.
NAW	24.1 bc	28.2 b	24.6 bc	22.1 bc	37.6 a	28.9 b	26.7 b	17.3 c	***	n.s.	***

Values with the same letter are not different according to Tukey's HDR test (P≤0.05).

 $\therefore$  \*\*\* and \*\* = significant at P  $\leq$  0.001 and 0.01, respectively. n.s. = not significant

Table 2. Effects of interaction "rate of GRH  $\times$  ADR treatment" on some chemical features of the substrates. As all interactions were significant, significance of the main factors are not reported. Symbols – or + refer to addition or not of ADR.

	GRH (%)												
Parameter	0	33	67	100	0+	33+	67+	100+	Sign.^				
рН	5.37 e	5.73 d	5.80 d	5.80 d	6.10 b	6.33 a	5.87 cd	6.07 bc	***				
$EC (mS cm^{-1})$	0.29 c	0.37 c	0.37 c	0.41c	1.10 a	0.67 b	0.61 b	0.61 b	***				
CEC (cmol kg <sup>-1</sup> )	141 a	80.9 c	46.2 e	28.5 f	112 b	82.0 c	57.1 d	36.5 ef	***				
TKN (%)	0.76 e	0.47 f	0.35g	0.27 h	1.97 a	1.76 b	1.29 c	1.01 d	***				
C/N ratio	56	96	133	180	20	25	35	45					
$NO_3-N (mg L^{-1})$	21.5 b	9.12 c	0.86 d	0.59 d	48.3 a	11.1 c	1.00 d	0.89 d	***				
$P (mg L^{-1})$	2.21 f	3.09 ef	5.55 de	9.44 ab	6.89 cd	8.31 bc	9.56 ab	11.0 a	*				
$K (mg L^{-1})$	12.0 c	34.1 bc	59.8 b	104 a	104 a	106 a	113 a	120 a	***				
Ca (mg $L^{-1}$ )	37.0 a	15.7 b	7.26 bc	3.67 c	32.6 a	13.0 bc	11.5 bc	7.26 bc	**				
Mg (mg $L^{-1}$ )	10.4 a	4.30 b	2.05 b	1.52 b	7.96 a	3.22 b	2.74 b	1.97 b	*				

Values with the same letter are not different according to Tukey's HDR test ( $P \le 0.05$ ).

^ significance of interaction (both rate of GRH and ADR treatment were significant at  $P \le 0.001$ ): \*\*\*, \*\* and \*= significant at  $P \le 0.001$ , 0.01 and 0.05 respectively.

		GRH	H (%)		AI	DR	Significance <sup>^</sup>			
Parameter	0	33	67	100	-	+	GRH	ADR	GRH×ADR	
OM (%)	71.2 d	76.8 c	79.6 b	80.7 a	79.1 a	75.1 b	***	***	n.s.	
OC (%)	41.3 d	44.5 c	46.2 b	46.8 a	45.9 a	43.5 b	***	***	n.s.	
$Mn (mg L^{-1})$	0.012 d	0.059 c	0.109 b	0.221 a	0.098	0.103	***	n.s.	n.s.	

Table 3. Effects of rate of GRH and addition (+) or not (-) of ADR on some chemical features of substrates.

Values with the same letter are not different according to Tukey's HDR test ( $P \le 0.05$ ).

 $\therefore$  \*\*\* = significant at P  $\leq$  0.001. n.s. = not significant.

Table 4. Effects of GRH(A) and addition (+) or not (-) of ADR(B) in the substrates and irrigation regime (IR)(C) on tomato transplant growth.

	GRH (%)				ADR			IS ^			Significance <sup>^</sup>					
Parameter	0	33	67	100	-	+	225	450	900	А	В	С	AxB	AxC	BxC	AxBxC
Height (cm)	15.0 a	12.6 b	10.2 c	8.70 d	11.2 b	12.1 a	10.7 b	12.9 a	11.3 b	***	***	**	***	***	*	*
Leaf number	4.48 a	4.08 b	3.44 c	3.01 d	3.56 b	3.95 a	3.59 b	3.96 a	3.71 b	***	***	**	*	n.s.	*	n.s.
Fresh weight (mg)	711 a	512 b	330 c	259 d	406 b	500 a	395 b	542 a	423 b	***	***	**	***	***	*	*
Dry weight (mg)	46.6 a	34.8 b	23.3 c	19.1 c	28.3 b	33.6 a	31,3	34.6	26.9	***	*	n.s.	*	n.s.	n.s.	n.s.
Leaf area (cm <sup>2</sup> )	18.8 a	13.5 b	8.75 c	6.22 d	10.3 b	13.4 a	10.9	14.1	10.5	***	**	n.s	***	*	*	n.s.

Values with the same letter are not different according to Tukey's HDS test ( $P \le 0.05$ ).

 $\uparrow$ : \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant

# **Figures**



Fig. 1. Effects of interaction "rate of GRH × ADR treatment" on tomato seedlings height. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 2. Effects of interaction "rate of GRH × ADR treatment" on tomato seedlings fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 3. Effects of interaction "rate of GRH × ADR treatment" on tomato seedlings leaf area. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 4. Effects of interaction "rate of GRH × IR" on tomato seedlings height. (GRH = relative rate of ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Fig. 5. Effects of interaction "rate of GRH × IR" on tomato seedlings fresh weight. (GRH = relative rate of ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.

**Chapter IV** 

Rice hull-based substrates fertilized with anaerobic digestion residues for cutting propagation of rose (*Rosa xhybrida* 'La Sevillana') and geranium (*Pelargonium hederifolium* 'Ville de Paris')

# Abstract

Vegetative propagation success in nursery production is affected by many factors including the plant species and rooting media. Rooting substrates are generally composed of peat amended with other inorganic components and fertilizers to achieve an adequate balance between water and air content and nutrients availability. In order to reduce peat use and exploit two agro-industrial byproducts some substrates were tested as rooting media for cutting production. Four substrates were prepared mixing sphagnum peat based substrates and ground rice hulls (GRH) (0, 33, 67 and 100% by volume of GRH). Four more substrates were then obtained by adding anaerobic digestion residues (ADR) of vegetal biomasses to each of them (20% by volume). Substrates were physically and chemically characterized and then tested as rooting substrates for Rosa ×hybrida 'La Sevillana' and Pelargonium hederifolium 'Ville de Paris' cuttings. Physical characteristics were strongly affected by increasing rate of GRH in substrates, which increased air filled porosity but seriously decreased available water, easily available water and water buffer capacity. ADR also affected physical characteristics, partly counteracting the negative effects of GRH. Chemical characterization pointed out that GRH addition results in a potential nutritional imbalance by reducing nitrate nitrogen and increasing P and K availability in the water extract and that ADR increase nutrient levels. Two weeks after cutting, rose cuttings were negatively affected by 100% of GRH, which reduced root growth and, at the end of the experiment, increasing GRH rates resulted in a gradual reduction of the overall cutting growth. On the other hand, in general GRH did not affect the geranium rooting process. Addition of ADR improved rose cuttings growth while it was negative for geranium. In conclusion, it is possible to save 46.4% of peat using the lower rate of GRH and 20% of ADR for rooting of rose cuttings and up to 100% using 100% of GRH for geranium propagation without reducing cuttings production and quality.

## Introduction

Substrates play an important role for rooting of cuttings (Atangana et al., 2006). They should present an adequate equilibrium between water and air content to assure water for cuttings uptake and supply sufficient aeration for adventitious rooting (Hartmann et al., 2002). In fact, rooting substrates are generally composed of an organic component, mostly peat, and an inorganic component (Sabalka, 1986) such as perlite or vermiculite, in order to improve physical properties i.e., increasing aeration.

However, there is no universal rooting medium for cuttings because the features required depend on the species, type of cutting, propagation system and season of cutting (Hartmann et al., 2002). For instance, Baldcypress cuttings rooted better in wet substrates (Copes and Randal, 1993; King et al., 2011) even if callus formation was better in aerated substrates (King et al., 2011). On the contrary, cuttings of some woody ornamental species rooted in pomace-based substrate performed better when amended with perlite, compared to bark and peat, due to the higher air-filled porosities (Chong and Dale, 2004). Fig tree cuttings also rooted better in substrates with perlite or peat + perlite (1:1) compared to sawdust or soil (Sirin et al., 2010).

Peat represents the largest constituent in growing media for containerized plant production because of its good physico-chemical characteristics such as high porosity, high water-holding capacity and relatively high cation-exchange capacity (Li et al., 2009). Environmental concerns, due to wetland conservation (Barkham, 1993; Robertson, 1993), and European laws (Gallagher, 2008) have limited peat availability resulting in a rise in its price (Ostos et al., 2006). This has increased interest in cheap and locally available alternative materials within a sustainable approach (Ribeiro et al., 2007; Marianthi, 2006; Iglesias-Díaz et al., 2009). Sawdust mixed with sand or gravel was used in some experiments for woody species propagation (Ofori et al., 1996; Tchoundjeu et al., 2002; Atangana et al., 2006; Mésen et al., 2007). Stoven and Kooima (1999) used fine bark and coconut coir mixed with perlite. Hazelnut cuttings rooted in pumice gave better results than those rooted in sand (Talaie and Nejatie, 1999). Chong and Dale (2004) tested grape pomace media amended with composted bark, perlite or peat. Erwin and Schwarze (1992) obtained better results rooting Clematis cuttings in sand or perlite than in substrates mixed with peat.

Since rice is one of the major crops in the world (FAO, 2009) rice hulls, its byproduct, are available in large volumes (Savita and Kamath, 1998). Rice hulls have been studied by several authors under different aspects as potential material for growing media. From the physical standpoint Kampf and Jung (1991) observed that carbonized rice hulls improved air porosity of substrates with peat. Garcia-C. et al. (2001) detected a higher

water holding capacity in composted rice hulls, compared to the fresh ones, even if it was still lower than advisable. Sambo et al. (2008) and Buck and Evans (2010) found that ground rice hulls (GRH) present physical properties much closer to those of peat. Chemical characteristics of rice hulls were studied by Gachukia and Evans (2008) and Evans and Gachukia (2008). Under the agronomical aspect, herbaceous and woody ornamental plant grown in substrates with rice hulls presented good growth (Laiche and Nash, 1990; Garcia-C. et al., 2001; Marianthi, 2006), while fresh and parboiled rice hulls appeared to be a reliable perlite alternative for growing herbaceous ornamentals (Papafotiou et al., 2001; Evans and Gachukia, 2004). Tomato production appears to be possible without yield and quality losses (Snyder, 1994), while more caution is required for pepper (Del Amor and Gómez-López, 2009). Parboiled rice hulls use is recommended to avoid soil-borne diseases, rice seed germination and release of toxic levels of Mn (Einert, 1972).

Anaerobic digestion is a process that decomposes organic matter producing biogas and can be applied to a wide range of feedstocks including industrial and municipal waste waters, agricultural, municipal, food industry wastes and plant residues (Ward et al., 2008). Anaerobic digestion residues (ADR) can be used in agriculture as fertilizers or amendments because they generally contain organic matter and high nutrient levels available for plants (Salminen et al., 2001; Tambone et al., 2008) after separation into a liquid and a solid fraction (Ward et al., 2008). Compton and Zauche (2006a; 2006b) obtained good results growing ornamental plants using anaerobic digestion biosolids derived from cattle manure. Unfortunately little information is available about the use of anaerobic digestion residues derived from vegetal byproducts as fertilizer in substrates.

The aim of this study was to evaluate the chemical, physical properties and agronomic performance of some substrates containing GRH and ADR as partial components of rooting substrates for rose and geranium cuttings.

## Material and Methods

#### Substrates preparation

Four substrates, named 0-, 33-, 67- and 100-, were prepared mixing peat with, respectively, 0, 33, 67 and 100% of 2 mm GRH, by volume. Four more substrates (0+, 33+, 67+ and 100+) were prepared fertilizing these mixes with ADR deriving from fruit distillery wastes (20% by volume).

#### Physical properties of substrates

EN13040 (1999) procedure was adopted for bulk density (BD) determination For substrates water retention curves determination, a pressure plate system was used (Tempe cell 1400, Soil Moisture Equipment Corporation, Santa Barbara, USA). Four samples for each substrate were saturated with deaerated and deionized water and weighed. Substrates were then subjected to increasing pressures of -1, -2, -5 and -10 kPa. Sample weights were recorded each time pressure systems reached equilibrium status to determine water volume retained at the different pressures. According to Fonteno and Bilderback (1993), the sum of all the water held on the tempe cell was considered total pore space (TPS). Water volumes held at pressures between 0 and -1 kPa and at -1 kPa were considered air-filled porosity (AFP) and water holding capacity (WHC) respectively. Available water (AW), easily available water (EAW) and water buffering capacity (WBC) were considered as water held at pressures between -1 and -10 kPa, between -1 and -5 kPa (De Boodt and Verdonck, 1972; Bunt, 1988) and between -5 and -10 kPa (Bruckner, 1997), respectively. According to Ingram et al. (1993), water released at pressure higher than 10 kPa was considered unavailable water (UW).

#### **Chemical properties of substrates**

Substrates pH were measured in 1:5 substrate-water suspension, while electrical conductivity (EC) was determined on 1:5 substrate-water extract according to EN 13037 and EN 13038 methodologies, respectively. Organic carbon (OC) and organic matter (OM) were determined after ashing 5 g of substrates using EN 13039 methodology. Dry matter (DM) of substrates was determined adopting the EN 13040 procedure. BaCl2-
triethanolamine methodology was used for cation exchange capacity (CEC) determination (Lax et al., 1986). Total Kjeldahl nitrogen (TKN) was also measured. Even if it underestimates total nutrient content, water extraction with deionized water (EN 13652) was preferred to other official methods (e.g. EN 13651) to obtain a measure of nutrients promptly available to plants. Hence nitrate-nitrogen (NO3-N), ammonium-nitrogen (NH4-N), P, K, Ca, Mg and S were evaluated by using ion chromatography (ICS-900, Dionex, Sunnyvale, CA, USA). Each analysis was performed on three samples per substrate.

## Agronomic evaluation of substrates

Four plastic trays with 72 cells (1600 mm<sup>2</sup> and 48620 mm<sup>3</sup> per cell) for each substrate were used for rooting cuttings of Rosa ×hybrida 'La Sevillana' and Pelargonium hederipholium 'Ville de Paris'. Initial cutting characterization is reported in table 1. Singlenode softwood cuttings were used for rose. Trays were placed in a greenhouse under a plastic tunnel to maintain high humidity with an initial mist frequency of 10 seconds 9 times per day. Temperature in this period ranged from 19 to 39 °C. Sixteen days after the experiment began a first sampling was performed. On 8 cuttings per tray, the presence of callus formation was noted, and stem diameter, total roots length, fresh and dry weight of root, stem, shoot and leaves were evaluated. Then, percentage of rooted cuttings, dry matter percentage and dry matter partitioning of different organs and total of plant, were determined, and root length-dry weight ratio, shoot-root ratio calculated. Lastly, the total dry matter was analyzed for TKN content. From this moment on, mist frequency was gradually reduced to 5 seconds 3 times per day from day 24 to day 30, and then misting was suspended. At this time cuttings were transferred into a plastic high-tunnel to complete the cropping cycle. Starting from the 4<sup>th</sup> week fertigations were applied 3 times per week using a 15N-2.2P-20.8K plus microelements hydrosoluble fertilizer. Electrical conductivity of the nutrient solution was raised during the cropping cycle from 1.2 to 1.5 mS cm<sup>-1</sup> from the 5<sup>th</sup> week (initial water EC =  $0.400 \text{ mS cm}^{-1}$ ). Different water requirements of cuttings/substrate were adjusted with manual irrigation with plain water according to the "look and feel" method (Niederholzer and Long, 1998) and Raviv and Lieth (2007). In order to improve cutting quality, new shoots were pinched above the third newly formed leaf. During this second phase, temperature ranged from 17 to 40 °C. The experiment was concluded after 56 days of cultivation, when cuttings of the best performing treatment reached commercial standard. At this moment, percentage of rooted cuttings was determined and, on 8 of them, stem and shoot diameter, shoot length, SPAD value (SPAD 502, Konica-Minolta, Japan), fresh and dry weight of root, stem, shoot, old leaf, new leaf were evaluated. Lastly, dry matter percentage of different organs, plant total and dry matter partitioning were determined and the total dry matter was analyzed for TKN content.

In the same greenhouse set-up an analogous experiment was conducted with Geranium; shoot-tip herbaceous cuttings were used for this species. Initial cutting characterization is reported in table 1. Mist frequency was 10 seconds 6 times per day for the first 4 days and then reduced to 4 times per day up to day 13. Cuttings were then fertigated 3 times per week using a 15N-2.2P-20.8K plus microelements hydro-soluble fertilizer. Electrical conductivity of the nutrient solution was 1.2 mS cm<sup>-1</sup> from day 14 to 20 and then raised to 1.5 mS cm<sup>-1</sup> from day 21 to the end of the trial. Different water requirements of cuttings/substrate were adjusted with manual irrigation with plain water according to the "look and feel" method (Niederholzer and Long, 1998) and Raviv and Lieth (2007). During the experiment temperature ranged from 14 to 35 °C. The experiment was concluded after 27 days of cultivation when cuttings of the best performing treatment reached commercial standard. Shoot diameter, shoot height, number of expanded and unexpanded leaves, SPAD value, fresh and dry weight of root, shoots and leaf were evaluated. Dry matter percentage of different organs, plant total and dry matter partitioning were also determined and the total dry matter was analyzed for TKN content.

### Data statistical analysis

Data were analyzed by analysis of variance (ANOVA) and means separated according to Tukey's HSD test ( $P \le 0.05$ ). Data were analyzed as a factorial experiment in a two way completely randomized design. Values expressed as percentages were transformed prior to ANOVA analysis.

## **Results and Discussion**

### Physical properties of substrates

Bulk Except for TPS, all physical properties were significantly affected by the main factors (relative rate of GRH and ADR in the substrates) and interaction "GRH  $\times$  ADR". For this reason, with the exception of TPS, only the "GRH  $\times$  ADR" interaction will be discussed. All results are reported in table 2.

Substrates BD decreased with increasing GRH content depending on ADR treatment. In general, ADR addition raised BD (on average +12.9%) but differences between not fertilized and ADR-fertilized substrates decreased with increasing of GRH content (22.6, 18.9 and 12.2% in 0, 33 and 67% GRH, respectively). No differences were detected between 100- and 100+ treatments. According to Buck and Evans (2010), some issues concerning settling and packing of particles, in particular when three components are mixed in a substrate rather than two, may explain the different trend observed with the ADR-fertilized compared to ADR-free substrates. BD values were in the range (190-700 g·cm<sup>-3</sup>) proposed by Bilderback et al. (2005) and Yeager et al. (2007) for containerized crop growing media.

TPS was affected only by GRH percentage in substrates. No differences were observed between substrates with 0 and 33% GRH (on average 90.2 and 91.6% of total volume). With 67% GRH, TPS decreased to 85.1% and was further decreased in 100% GRH substrates (80.7%). Substrates with 67% GRH had ideal TPS value (De Boodt and Verdonck, 1972), however all substrates presented acceptable values (Arnold Bik, 1983; Boertje, 1984; Bilderback et al., 2005).

In substrates without ADR, AFP increased with increasing of GRH by 54.8% (100vs. 0-). In general, ADR-fertilized substrates had lower AFP than unfertilized substrates (on average, -16.8%) and, here, no differences were observed in substrates containing 33% or more of GRH. As AFP values for rooting substrate should range from 10 to 40% (Maronek et al., 1985; Aendekerk; 1993 ) only 0- and 0+ substrates are satisfactory for this parameter. All the other values were to high as a result of the addition of GRH, which is known for its high AFP. This characteristic may promote callus formation but may be unfavorable for rooting (King et al., 2011). In unfertilized substrates, WHC was strongly reduced by the increasing amount of GRH (-67.4%; 100- vs. 0-) while ADR addition improved WHC to a higher extent in 100% GRH substrates (96.2%) than in 0% GRH substrates (12.2%). Maronek et al. (1985) proposed a WHC ranging from 20 to 60% as adequate for rooting media. Except for 100-, all tested substrates were within these values. The low WHC of 100- substrate is probably a consequence of its high AFP. However other authors indicate, for horticultural substrates, a range from 50 to 75% for WHC. Considering these, only 0- and 0+ presented optimal values (Bunt, 1988; Handreck and Black, 2002).

AW and EAW had a similar response pattern to WHC but ADR addition improved only values of substrates with 67 and 100% GRH (+62.9 and +134.4% for AW and +41.4 and +166.7% for EAW, respectively). As the optimal range for AW and EAW are 23-35 % and 20-30%, all substrates had low values (Bilderback et al., 2005; De Boodt and Verdonck, 1972). However the substrates with characteristics closer to the recommended values are those GRH-free.

WBC decreased by 72.7% with increasing rate of GRH in the substrates (100% vs. 0% GRH substrates). ADR addition increased WBC only in substrates with 67% GRH (2.7 times higher). Except for 0- and 0+, values again appeared lower than recommended (4-10%; De Boodt and Verdonck, 1972).

UW decreased by 53.7% increasing GRH content from 0 to 100% and values were raised by ADR addition (20.3, 23.1, 42.1 and 78.4% in substrates with 0, 33, 67 and 100% GRH, respectively). Bilderback et al. (2005) suggested normal values should range between 23 and 35%. The values obtained in this experiment seem adequate only in substrates with 0 and 33 GRH, while the other substrates revealed low values.

High AFP values at the expense of WHC in substrates containing rice hulls were observed previously (Evans and Gachukia, 2007; Evans et al., 2011). It is not surprising that AW, EAW and WBC values also decrease with the increase of GRH. However, the optimum values range is often referred to a general potting substrate and not to the particular case of rooting substrates, which generally require more AFP. The obtained values suggest that, in substrates containing GRH, water should be applied frequently and in small quantities (Benito et al., 2006), which might be met, in the case of cuttings, by means of the mist treatment.

## Chemical properties of substrates

In some cases, the analysis of variance performed on chemical characteristics indicated significant effects only of main factors (Table 3) and, in others, also the significance of interaction (Table 4).

EC values were affected only by main factors (Table 3). Significant differences were observed between 100% GRH and the other mixes, with EC values 15.6% higher, but within the normal range for EC. ADR-fertilized substrates had EC levels 65.6% higher than the ADR-free ones. According to Handreck and Black (2002) and Zaccheo (2009), low EC levels, ranging between 0.12 and 0.35 mS cm-1, are suitable for bedding plants and plants sensitive to salinity, while normal EC levels of 0.36-0.65 mS cm-1 are in general acceptable for any pot plant production. The data obtained here indicate that these substrates are more suitable for pot production.

OM and OC (Table 3) increased from 56.1 to 79.6% and from 31.6 to 46.2% respectively as GRH increased from 0 to 100%. The addition of ADR to the substrates reduced OM and OC values by 8.9%. This is due to the industrial process that produced gas at the expense of organic matter (Ward et al., 2008). OM and OC of 100% GRH are consistent with those observed by Zanin et al. (2011) and are close to those considered advisable by Abada et al. (2001); while all the other substrates had values lower than advisable, probably because the starting material was a commercial peat-based substrate.

Percentage of TKN (Table 3) decreased by 77.2% as the rate of GRH increased from 0 to 100%. In general, ADR fertilized substrates had 3.22 times higher TKN compared to those ADR-free. TKN content in the control was a little lower than that suggested by Bunt (1988), however values obtained are similar to those of sphagnum peat and commercial peat-based substrates used by Benito et al. (2006).

Concentration of P in the water extract (Table 3) increased with increasing GRH content from 3.6 to 5.0 mg/L (0 vs. 100% GRH) and fertilizing substrates with ADR (+19.1%). Values were lower than recommended (6.11-8.29 mg L-1; Pozzi and Valagussa, 2009).

Concentration of S (Table 3) decreased with increasing GRH content in the substrates. ADR addition raised S concentration, but values were nevertheless lower than recommended (29.05-37.35 mg L-1; Pozzi and Valagussa, 2009).

Substrate pH was affected by the interaction "GRH × ADR" (Table 4). Values rose with increasing of GRH percentage. Fertilization with ADR increased values, but the relative increment decreased as the GRH content increased (24.6, 18.5, 12.0 and 9.5% in substrates with 0, 33, 67 and 100% GRH, respectively). According to Maroneck et al. (1985), rooting substrates should have pH ranging from 4.5 to 6.5. Only 100+ substrates had higher values while, in particular 67-, 100-, 0+, 33+ and 67+, had optimal values (5.3-6.5; Abad et al., 2001).

Increasing GRH in substrates from 0 to 100%, CEC decreased by 65.8%. ADR addition reduced CEC of 0+ if compared to 0- (-12.8%) but no differences were observed with other substrates. CEC value obtained for the peat control is comparable with those found by other authors (Bunt, 1988; Lemaire, 1999; Benito et al., 2006). Decreasing CEC values are related to pH increase because overall peat presents a pH-dependent charge (Cattivello, 2009). Moreover high CEC levels in peat may also be due to higher levels of OM (Benito et al., 2006).

DM of substrates increased with the increasing GRH content in substrates from 31.6% in 0- substrate to 90.4% in 100- substrate. ADR fertilization increased DM by 5.3% in 0% GRH substrate while it decreased by 2.8, 10.6 and 13.1% in 33, 67 and 100% GRH substrates, respectively. These results are due to the mixing of raw materials that are characterized by different moisture content.

Concentration of NO3-N in the water extract decreased as GRH rate increased both in ADR-free and ADR-fertilized substrates, but values were higher in the latter mixes than in the former. NO3-N concentration appeared to be appropriate only for 0+ substrate and low in all the others (11-23 mg L-1; Pozzi and Valagussa, 2009).

Concentrations of NH4-N increased with GRH content and generally decreased with ADR fertilization. Concentration of this nutrient in 0- control showed significant differences only with 100-. In all cases values appeared markedly lower than acceptable (8-12 mg L-1; Pozzi and Valagussa, 2009).

Both GRH and ADR increased K concentration in the mixes. Values increased from 23.4 to 118 mg L-1 in ADR-free substrates, and from 71.9 to 135.3 mg/L in ADR-fertilized substrates. Levels for this nutrient appear to be too high (>14 mg L-1; Pozzi and Valagussa, 2009). The high levels of K in substrates containing rice hulls is well known and

particularly if ground (Cadell, 1988; Cattivello, 2009; Zanin et al., 2011).

Concentrations of Ca and Mg decreased with increasing GRH percentage in the substrates. ADR addition improved Ca levels at all GRH percentages, while those of Mg only in 33 and 67% GRH. Decreasing levels of these nutrients are related to decreasing levels of CEC due to the lower amount of peat in the substrates (Cattivello, 2009). According to Pozzi and Valagussa (2009), Ca concentrations are adequate only in 67+ substrate, while those of Mg only in 0-, 0+ and 33+ substrates.

#### Agronomic evaluation of substrates

Rose cuttings rooting experiment - first sampling. No "GRH  $\times$  ADR" interaction resulted as significant, so only main effects will be reported. All data are reported in table 5.

The percentage of cuttings with callus were close to 100% in all cuttings independently of the substrate used (data not shown). Instead, the percentage of rooted cuttings was affected by ADR treatment and fertilization with this material induced a significant increment from 40.6 to 67.2%. Stem diameter was also not affected by treatments and was 4.26 mm on average. Total root length of rooted cuttings decreased by 77.4% from 51.5 to 11.7 mm increasing GRH content from 0 to 100%. Total root length was improved by 60% when cuttings rooted in media with ADR. Fresh and dry weight of roots were lower (-77.6 and -69.5%, respectively) in substrates containing 100% GRH compared to those without GRH. ADR fertilization in substrates increased both root fresh and dry weight by 62.9 and 98.7%. Fresh and dry weight of stem, new shoot, old leaves and of the whole cutting were not affected by treatments and were, on average, 366, 67.5, 466, and 926 mg for the fresh weight and 109, 17.0, 156 and 284 mg for the dry weight (data not shown). Root dry matter was affected only by GRH and ranged from 10.6 to 17.5% in substrates with 0 and 100% GRH, respectively. Instead, stem and shoot dry matter decreased by 9.7 and 6.6% adding ADR to the substrates. Leaf dry matter was not affected by treatments and was 33.3% on average. Total dry matter of cuttings rooted in substrates without GRH was lower than those with 100% (on average -8.4%) but not significant differences were observed with intermediate GRH content. ADR fertilization decreased total dry matter by 4.9%. Partitioning of dry matter among different organs highlighted that roots were more highly represented in the total of cuttings rooted in 0% GRH than those in

100% GRH (+69.6%); ADR fertilization improved this parameter by 92.2%. Relative weight of stems, shoots and leaves were not affected by treatments and resulted on average as 38.6, 5.94 and 54.5%, respectively. TKN in the plant tissue was 1.45% independently of treatment. Root length:dry weight ratio decreased by about 44% in cuttings rooted in 100% of GRH compared to the control. Root-shoot ratio was not affected by treatments and was 171 on average.

Sixteen days after cutting some differences were observed only in parameters concerning roots. Chen et al. (2003) observed similar behavior in rooting pothos, maranta and schefflera and they reported that it was probably due to physical properties of the different substrates. Copes and Randall (1993) reported that excessive aeration negatively affected the rooting process and in this experiment rooting might be affected by high amounts of GRH which are characterized by high AFP. ADR addition increased nutrient levels and slightly improved root development. This is consistent with results obtained by Jonson (1977) and Wott and Tukey (1967).

*Rose cuttings rooting experiment - final sampling*. At the end of the experiment, rooting percentage decreased from 84.5 to 71.5 increasing GRH from 0 to 100%, and no differences were observed between 0 and 33% GRH and between 67 and 100% GRH (Table 6).

In the second sampling, again no "GRH  $\times$  ADR" interaction resulted as significant and only SPAD values and root fresh weight were affected by both relative percentage of GRH in the substrate and any addition of ADR (Table 6). Those parameters will be presented first. Parameters affected only by GRH relative rate will then be discussed and subsequently those affected by ADR fertilization.

Higher SPAD values (Fig. 1) were measured in plant cuttings rooted in GRH-free substrates. No differences were observed between cuttings in substrates with different GRH. SPAD values resulted as 13.1% higher when substrates were fertilized with ADR (Fig. 1). Root fresh weight (Fig. 2) was progressively reduced increasing relative rate of GRH in substrates (-28.4; 0 vs. 100% GRH) but not significant differences were detected in substrates with 67 and 100% GRH. Substrates fertilization with ADR induced 20.3% heavier fresh root weight (Fig. 2).

Cuttings rooted in 33% GRH had wider stem diameter than those rooted in 0%

GRH substrates; cuttings rooted in 33% GRH also had wider shoot diameter, but no statistical difference was observed in this with 0% GRH, while it was higher than cuttings rooted in the higher rates of GRH (Table 6).

Increasing relative GRH rate in substrates resulted in shorter shoots; substrates with 67 and 100% GRH presented similar values for this parameter (Table 6).

Root dry weight was improved by ADR fertilization, with values about 16% higher (Table 6).

Fresh and dry weights of old parts of cuttings (stem and leaf) responded to treatments in the same way. Shoot fresh and dry weight in rose rooted in 33% GRH were higher than those in 0% GRH (+37.4 and +42.0%, respectively). Old leaf fresh and dry weight in 33% GRH substrates were higher by 114 and 1230%, respectively). Cuttings rooted in substrates with 33% GRH showed no significant difference compared to those rooted at higher rates of GRH (Table 6). New shoot fresh and dry weight gradually decreased along with the increase in rate of GRH, with values in 100% GRH about 50% lower than those in 0% GRH. Similar results were observed for new leaves fresh and dry weight with, in this case, reductions of about 55%.

The negative effect on weights of new cutting organs were in part counteracted by a lower loss of weight of the old organs so that fewer difference were observed in fresh weight of the whole cuttings (-24.5 between cuttings rooted in 100% GRH compared to those in 0% GRH). No differences were observed in total dry weight (Table 6).

Percentage of dry matter of different cutting organs were minimally affected by treatments: roots of cuttings rooted in 100% GRH had a lower value compared to that in 0% GRH (-36.7%). Stem dry matter was higher in substrates containing ADR (+ 5.2%).

Dry matter partitioning among cutting organs, reported in figure 3, highlights that not many differences were observed among substrates containing GRH, and all had a higher relative dry matter accumulated in old stems and leaves and lower accumulation of dry matter in new shoots and leaves.

TKN in the cutting tissues decreased progressively and was 20.1% lower in the 100% GRH compared to that of the control (Table. 6).

Lastly, shoot:root ratio was reduced by 19.2% fertilizing the substrates with ADR.

At the end of the experiment, it was clear that GRH influenced a wider range of

parameters with respect to the first sampling date. In general, increasing percentage of GRH negatively affected rooted cutting growth and quality (e.g. SPAD, weight of different plant parts, biomass partitioning of different plant parts). Physical properties may have played an important role. Too high aeration may have delayed rooting process after callus formation and substrates with higher WHC (e.g. 0 and 33% GRH) may have favored root growth (King et al., 2011). Also Rein et al.(1991) underlined the importance of moisture in the propagation medium to produce adventitious roots. On the other hand, ADR addition did not generally affect rooting process and rooted cuttings.

*Geranium rooting experiment.* Rooting percentage of geranium cuttings ranged from 92.7 in 100- to 99.7 in 0+ without differences between studied treatments. In this experiment significant "GRH  $\times$  ADR" interactions were observed only in biomass partitioning among different cutting organs and TKN concentration in the tissues and only shoot diameter, root fresh weight, root dry weight and dry matter partitioning were affected by main factors (Table 7).

Geranium shoot diameter was negatively affected by ADR fertilization (-5.6%; Table 7) while plant height, expanded leaf number, unfolding leaf number, total leaf number and SPAD values were not affected by treatments and were on average 8.61 cm, 3.17, 2.16, 5.34 and 41.6 respectively.

Geranium root fresh weight resulted as lower in substrates with 0% GRH (178 mg) and higher in those with 67% GRH (297 mg) (Fig. 7). ADR addition to substrates reduced root fresh weight by 33.4% (Fig. 4). Root dry weight of cuttings rooted in 0% GRH substrates did not differ from those rooted in substrates with 33 and 100% GRH, but was higher in 67% GRH substrate (Fig. 5). ADR fertilization reduced this parameter by 32.6% (Fig. 5). The dry weight of shoot, leaf, aerial part and total cutting were not affected by treatments and resulted as on average 44.9, 150, 193 and 213 mg, respectively. Root, shoot leaf and total dry matter were also not affected by treatments and were on average 7.97, 9.70, 8.92 and 7.93%, respectively.

Dry matter partitioning among geranium cutting organs highlights that a higher accumulation of dry matter occurred in substrates containing 33 and 67% compared to the control, while fertilization with ADR reduced dry matter accumulation in roots (Fig. 6). In ADR-free substrates, dry matter accumulation in leaves was lower in substrates containing

GRH compared to the control (0-), while no differences were observed in substrates with ADR-fertilization (Fig 7).

Shoot:root ratio resulted as lower in substrates with 33 and 67% GRH if compared to those with 0% GRH, but no significant differences were observed comparing substrates with 0 and 100% GRH (Table 7).

TKN concentration in geranium cuttings decreased as GRH rate in the substrate increased but ADR addition sustained TKN in cuttings rooted in substrates with 33 and 67% GRH (Fig. 11).

In this experiment geranium cutting performance had very few differences despite the very different substrates used. Differences between substrates regarded mainly root growth where a lower root development was observed in substrates without GRH. Those substrates had a higher rate of WHC than the others and pH value lower than 5.5. Loehrlein and Craig (2004) reported that high WHC and pH values lower than 5.5 are not recommendable for geranium.

## Conclusions

GRH addition in substrates changed many physical properties, greatly increasing AFP and consequently reducing volume for AW, EAW and WBC. On the other hand, ADR addition limited this negative trend improving WHC, and partially AW, EAW and WBC. Under the chemical aspect, GRH addition increased DM, OM, CO, NH<sub>4</sub>-N, P and K while it reduced the other nutrient contents (N, Ca, Mg, S), pointing out a potential nutritional imbalance. ADR addition instead increased all nutrient contents and pH values in substrates. Rose and geranium rooting had different responses to increasing GRH rate in substrates and to ADR-fertilization. After 16 days from cutting, rose was already negatively affected by increasing GRH content in terms of root growth, at the end of the experiment this reduction involved several other parameters. Geranium cuttings were generally not affected by GRH, which improved some parameters compared to substrates with 0% GRH. ADR addition had a small positive effect on rose rooting in very early stage but this was less evident at the end of the experiment. Instead, when significant, the effect of ADR on geranium rooting was negative (e.g. lower root fresh and dry weight). In conclusion, under a sustainable approach, it is advisable to save 46.4% of peat by using 33% GRH and fertilizing with 20% of ADR in rooting cuttings of 'La Sevillana' rose. Instead, in rooting of 'Ville de Paris' geranium cuttings peat appears to be suitably totally substituted by GRH without fertilization with ADR.

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# Tables

	Rose	Geranium
Stem diameter (mm)	3.51	2.71
Stem length (mm)	24.2	53.7
Expanded leaf (n°)	1	1.30
Expanding leaf (n°)	-	2.20
Leaf fresh weight (mg)	316	793
Stem fresh weight (mg)	222	345
Total fresh weight (mg)	538	1138
Leaf dry weight (mg)	94	84
Stem dry weight (mg)	66	26
Total dry weight (mg)	160	110
Leaf dry matter (%)	30.2	10.8
Stem dry matter (%)	26.9	7.61
Total dry matter (%)	29.4	9.77

Table 1. Initial characterization of rose and geranium cuttings.

Table 2. Effects of "GRH (A)  $\times$  ADR (B)" interaction on bulk density (BD) total pore space (TPS), air-filled porosity (AFP), water holding capacity (WHC), available water (AW), easily available water (EAW), water buffer capacity (WBC) and unavailable water (UW) of the substrates (values indicate % by volume).

	Substrate									Significance <sup>^</sup>		
	0-	33-	67-	100-	0+	33+	67+	100+	А	В	A×B	
BD $(g \cdot cm^{-3})$	0.349 c	0.317 de	0.295 f	0.311 ef	0.428 a	0.377 b	0.331 cd	0.301 ef	***	***	***	
TPS (%)	90.7	91.3	84.9	80.7	89.7	91.9	85.2	80.7	***	n.s.	n.s.	
AFP (%)	41.9 e	55.3 c	61.2 b	64.8 a	35.0 f	51.0 d	50.1 d	49.5 d	***	***	***	
WHC (%)	48.8 b	36.0 d	23.8 f	15.9 g	54.8 a	40.9 c	35.1 d	31.2 e	***	***	***	
AW (%)	18.8 a	13.1 b	6.37 d	4.91 d	18.6 a	12.8 b	10.4 c	11.5 bc	***	***	***	
EAW (%)	14.2 a	10.38 b	5.28 d	3.82 d	14.4 a	9.83 b	7.46 c	10.2 b	***	***	***	
WBC (%)	4.63 a	2.70 b	1.09 c	1.09 c	4.26 ab	2.92 b	2.91 b	1.33 c	***	**	**	
UW (%)	30.0 b	22.9 c	17.4 d	11.0 e	36.1 a	28.2 b	24.7 c	19.7 d	***	***	**	

 $\therefore$  \*\*\*, \*\* and \* = significant at P  $\le$  0.001, 0.01 and 0.05, respectively. n.s. = not significant.

	GRH (%)					ADR			Significance <sup>^</sup>		
Parameter	0	33	67	100		-	+	GRH	ADR	GRH×ADR	
$EC (mS cm^{-1})$	0.412 b	0.413 b	0.412 b	0.477 a		0.323 b	0.534 a	***	***	n.s.	
OM (%)	56.1 d	68.7 c	75.2 b	79.6 a		73.2 a	66.6 b	***	***	n.s.	
OC (%)	32.6 d	39.9 c	43.6 b	46.2 a		42.4 a	38.7 b	***	***	n.s.	
TKN (%)	0.86 a	0.49 b	0.33 c	0.20 d		0.22 b	0.71 a	***	***	n.s.	
$P(mg L^{-1})$	3.59 c	3.77 bc	3.95 b	4.95 a		3.71 b	4.42 a	***	***	n.s.	
S (mg L <sup>-1</sup> )	17.4 a	10.5 b	5.39 c	2.24 d		7.65 b	10.1 a	***	***	n.s.	

Table 3. Effects of relative rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on some chemical features of the substrates (EC is the electrical conductivity, OM is the organic matter, OC is the organic carbon and TKN is the total Kjeldhal nitrogen).

 $\therefore$  \*\*\* and \*\* = significant at P  $\le$  0.001 and 0.01 respectively. n.s. = not significant

	Substrate								
Parameter	0	33	67	100	0+	33+	67+	100+	Sign.^
рН	4.60 f	5.03 e	5.57 d	6.20 b	5.73 d	5.97 c	6.23 b	6.77 a	***
CEC (meq 100g)	145 a	89.1 c	62.0 d	44.7 e	126.1 b	86.5 c	67.3 d	49.4 e	***
DM (%)	31.6 g	49.6 e	72.6 c	90.4 a	33.2 g	48.2 f	65.0 d	78.5 b	***
$NO_3-N (mg L^{-1})$	9.89 b	6.44 c	3.23 e	0.73 f	12.34 a	9.22 b	6.58 c	4.79 d	*
$NH_4$ -N (mg L <sup>-1</sup> )	0.40 bcd	0.30 d	0.67 ab	0.88 a	0.34 cd	0.48 bcd	0.58 bcd	0.62 ab	*
$K (mg L^{-1})$	23.4 f	54.4 e	84.6 cd	117.7 b	71.9 d	98.5 c	120.1 ab	135.3 a	**
$Ca (mg L^{-1})$	20.33 b	8.84 d	2.72 e	1.55 e	31.42 a	21.52 b	13.59 c	9.51 d	*
Mg (mg $L^{-1}$ )	9.89 a	5.14 c	2.60 d	2.31 d	9.67 a	6.81 b	4.48 c	2.56 d	**

Table 4. Effects of "GRH × ADR" interaction on chemical features of the substrates (CEC is the cation exchange capacity, DM is the dry matter).

 $^{\circ}$  significance of interaction (both rate of GRH and ADR treatment were significant at P $\leq$ 0.001): \*\*\* and \*\* = significant at P  $\leq$  0.001 and 0.01 respectively.

-		GRH	H (%)	AI	OR	Significance <sup>^</sup>			
Parameter	0	33	67	100	-	+	GRH	ADR	GRH×ADR
Rooted cuttings (%)	71.9	56.3	46.9	40.6	40.6 b	67.2 a	n.s.	**	n.s.
Total root length (mm)	51.5 a	34.0 ab	34.7 ab	11.6 b	25.4 b	40.6 a	**	*	n.s.
Root fresh weight (mg)	40.2 a	24.2 ab	30.2 a	9.0 b	19.7 b	32.1 a	**	*	n.s.
Root dry weight (mg)	3.68 a	2.51 ab	3.13 a	1.12 b	1.73 b	3.43 a	**	**	n.s.
Root dry matter (%)	10.6 b	12.6 ab	11.1 b	17.5 a	13.7	12.2	**	n.s.	n.s.
Stem dry matter (%)	28.9	30.1	30.1	30.4	31.4 a	28.4 b	n.s.	***	n.s.
Shoot dry matter (%)	25.9	27.5	27.2	28.2	28.1 a	26.3 b	n.s.	*	n.s.
Cutting total dry matter (%)	29.5 b	31.0 ab	31.4 ab	32.0 a	31.7 a	30.2 b	*	**	n.s.
Root relative weight (%)	1.35 a	0.95 ab	1.03 ab	0.41 b	0.64 b	1.23 a	*	**	n.s.
Root length:dry weight ratio	16.6 a	13.3 ab	11.6 ab	9.3 b	13.7	11.7	*	n.s.	n.s.

Table 5. Effects of relative rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on rose cuttings at the first sampling.

 $\uparrow$ : \*\*\* and \*\* = significant at P  $\leq$  0.001 and 0.01 respectively. n.s. = not significant

	GRH (%)			A	DR	Significance <sup>^</sup>			
Parameter	0	33	67	100	-	+	GRH	ADR	GRH×ADR
Rooted cuttings (%)	84.5 a	82.1 a	75.4 b	71.4 b	78.3	78.4	***	n.s.	n.s.
Stem diameter (mm)	4.26 b	4.95 a	4.56 ab	4.49 ab	4.43	4.69	**	n.s.	n.s.
Shoot diameter (mm)	2.24 ab	2.34 a	2.16 b	2.16 b	2.21	2.21	***	n.s.	n.s.
Shoot length (cm)	10.59 a	8.06 b	6.01 c	5.30 c	7.69	7.29	***	n.s.	n.s.
Stem fresh weight (mg)	382 b	525 a	514 a	551 a	495	492	**	n.s.	n.s.
Shoot fresh weight (mg)	393 a	325 ab	233 bc	201 c	300	276	***	n.s.	n.s.
Old leaf fresh weight (mg)	188 b	402 a	417 a	425 a	362	354	***	n.s.	n.s.
New leaf fresh weight (mg)	1370 a	943 b	695 b	595 b	858	943	***	n.s.	n.s.
Total fresh weight (mg)	2873 a	2679 ab	2283 ab	2144 b	2426	2563	*	n.s.	n.s.
Root dry weight (mg)	74.2	77.0	68.1	69.0	65.7 b	78.5 a	n.s.	*	n.s.
Stem dry weight (mg)	119 b	169. a	162 a	172 a	153	158	**	n.s.	n.s.
Shoot dry weight (mg)	97.8 a	78.0 ab	56.8 bc	48.7 c	70.4	70.2	***	n.s.	n.s.
Old leaf dry weight (mg)	56 b	125 a	139 a	140 a	117	113	***	n.s.	n.s.
New leaf dry weight (mg)	357 a	254 b	179 bc	159 c	220	254	***	n.s.	n.s.
Root dry matter (%)	12.0 b	13.7 ab	15.1 ab	16.4 a	14.6	14.0	*	n.s.	n.s.
Stem dry matter (%)	31.2	32.7	31.7	31.0	30.8 b	32.5 a	n.s.	*	n.s.
TKN (%)	1.72 a	1.37 b	1.19 c	1.12 c	1.34	1.37	***	n.s.	n.s.
Shoot:root ratio	9.20	8.36	8.43	9.42	9.79 a	7.91 b	n.s.	**	n.s.

Table 6. Effects of relative rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on rose cuttings at the end of the experiment.

 $\uparrow$ : \*\*\* and \*\* = significant at P  $\leq$  0.001 and 0.01 respectively. n.s. = not significant

Table 7. Effects of relative rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on geranium cuttings at the end of the experiment.

		ADR		Significance <sup>^</sup>					
Parameter	0	33	67	100	-	+	GRH	ADR	GRH×ADR
Shoot:root ratio	18.7 a	11.6 b	8.9 b	12.0 ab	11.1	14.5	**	n.s.	n.s.
Shoot diameter (mm)	2.86	2.99	2.97	3.03	3.05 a	2.88 b	n.s.	*	n.s.
Shoot (%)	21.7	20.2	19.3	20.7	19.6 b	21.4 a	n.s.	**	n.s.
Leaf (%)	71.9 a	69.7 ab	68.8 b	70.2 ab	69.7	70.6	*	n.s.	*
TKN (%)	3.09 a	2.71 b	2.67 bc	2.47 c	2.63b	2.83 a	***	**	***

 $\uparrow$ : \*\*\* and \*\* = significant at P  $\leq$  0.001 and 0.01 respectively. n.s. = not significant

# **Figures**



Figure 1. Effect of rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on rose SPAD value at the final sampling. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 2. Effect of rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on rose root fresh weight (mg) in the final sampling. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 3. Effect of rate of ground rice hulls (GRH) on rose dry matter partitioning among rose cutting organs at the final sampling. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure4. Effect of rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on geranium root fresh weight (mg). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 5. Effect of rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on geranium root dry weight (mg). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 6. Effect of rate of ground rice hulls (GRH) and addition (+) or not (-) of anaerobic digestion residues (ADR) on relative content of root dry matter in the total dry matter of geranium cuttings. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



**GRH content in substrate (%)** 

Figure 7. Effect of "GRH x ADR" interaction on relative content of leaves dry matter in the total dry matter of geranium cuttings. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 8. Effect of "GRH x ADR" interaction on TKN concentration in geranium cuttings. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.

**Chapter V** 

Rice hull-based substrates fertilized with anaerobic digestion residues for potted geranium (*Pelargonium hederipholium* 'Ville de Paris') and rose (*Rosa ×hybrida* 'La Sevillana')

## Abstract

Economic and environmental concerns have limited peat use for substrates production promoting interest in alternative materials. The aim of this study was to determine the physical and chemical characteristics of substrates with alternative materials to peat and evaluate their effect on production of potted geranium and rose.

Four substrates were prepared mixing sphagnum peat-based substrates with unground rice hulls (URH) (0, 33, 67 and 100% by volume of RH). Four more substrates with these ingredients and proportions were fertilized with anaerobic digestion residues (ADR) of vegetal biomasses (20% by volume). Eight further substrates were prepared as above substituting URH with 2 mm of ground rice hulls (GRH). Substrates were physically and chemically characterized and then tested as potting substrates for *Pelargonium hederipholium* 'Ville de Paris' and *Rosa* ×*hybrida* 'La Sevillana' production.

Physical characteristics (in particular air-filled porosity and water holding capacity) worsened increasing RH content. Some substrates with high RH content exceeded optimal range limits. This problem was partly solved using GRH and in a minimal part by fertilizing substrates with ADR.

Chemical characteristics were affected especially by RH rate in the substrate and by ADR fertilization. RH increased P and K availability in the water extract, reducing CEC, nitrogen percentage, NO<sub>3</sub>-N Ca and S causing a possible nutritional imbalance. ADR addition instead increased all nutrients, restoring nutritional equilibrium.

In the cultivation experiments geranium and rose resulted as negatively affected by increasing rate of RH which involved several biometrical parameters probably because of worsening of physical properties of substrates. ADR fertilization of substrates improved many biometrical parameters in geranium but did not affect those of rose. In both species GRH use instead of URH improved some considered parameters. It resulted as being possible to partly substitute peat with 33% RH, but GRH plus ADR fertilization are necessary for geranium production, while this recommendation is facultative for rose. GRH use and ADR fertilization are also recommended in order to reduce water volume for plants and destroy rice seedlings.

## Introduction

Because of its high porosity, high water-holding capacity and relatively high cationexchange capacity peat is the most important ingredient for media production (Li et al., 2009) But its wide exploitation has raised interest in wetland conservation (Barkham, 1993; Robertson, 1993) and European laws (Gallagher, 2008) have been passed that limited peat availability and increased its price (Ostos et al., 2008), especially in southern European countries that import peat (Ribeiro et al., 2007). For these reasons many authors have reported an increasing interest in finding cheap and locally available alternative materials with a sustainable approach (Ribeiro et al., 2007; Marianthi, 2006; Iglesias-Díaz et al., 2009). Reducing water and fertilizer use are also important for achieving sustainable container production system (Biernbaum, 1992; Uva et al., 1998). The alternative materials should therefore have suitable physical and chemical properties, uniform quality, be available in large quantities and cost effective (Evans et al., 1996; Fecondini et al., 2009). Since no universal substrate exists, many materials are mixed with peat to achieve desirable physical and chemical properties (Bunt, 1971; Fonteno, 1993; Bachman and Metzger, 2007).

Rice hulls are an interesting rice byproduct that can be used as a component of substrates and are readily available in large quantities (Savita and Kamath, 1998; Del Amor and Gomez-Lopez, 2009) as they represent 20% of the weight of harvested rice. They have been studied under different aspects since the 70s as potential material for growing media. The use of parboiled rice hulls has been recommended to avoid soil-borne diseases, rice seed germination and release of toxic levels of Mn (Einert, 1972). Carbonized and composted rice hulls improve air porosity of substrates (Kampf and Jung, 1991; Garcia-C. et al., 2001) reducing water holding capacity (Garcia-C. et al., 2001), while ground rice hulls presented physical properties closer to peat (Sambo et al., 2008; Buck and Evans, 2010). Fresh and ground rice hull chemical properties have also been studied (Kampf and Jung, 1991; Gachukia and Evans, 2008; Evans and Gachukia, 2008; Zanin et al., 2011). In cultivation experiments good plant growth was obtained cultivating woody ornamentals (Laiche and Nash, 1990; Marianthi, 2006) and herbaceous ornamentals (Garcia-C. et al., 2001), while different results depending on cultivated species were obtained with vegetables (Snyder, 1994; Del Amor and Gómez-López, 2009; Zanin et al., 2011). Rice

hulls represent a good perlite alternative for growing ornamental foliage (Papafotiou et al., 2001) and flower plants (Evans and Gachukia, 2004).

Anaerobic digestion is an interesting disposal method for fresh biomass waste that may bring numerous environmental and economic advantages (Braber, 1995) and it can be applied to a wide range of biomasses (e.g. plant residues, sewage sludge, the organic fraction of municipal wastes, agricultural byproducts) (Ward et al., 2008). Because of their low C/N ratio, high nutrient levels, and organic matter, anaerobic digested residues can be used in agriculture as fertilizers or amendments (Salminen et al., 2001; Tambone et al., 2008) after separation by liquid fraction (Ward et al., 2008). Good geranium and orchid plants were obtained using anaerobic digestion biosolids of cattle manure (Compton and Zauche, 2006a; Compton and Zauche, 2006b).

Some experiments demonstrated the possibility of cultivating several foliage or flower ornamentals in substrates with a partial substitution by compost made with different biomass (e.g. cattle manure, grape marc, green compost and sewage sludge) (Chen et al., 1988; Grigatti et al., 2007; Caballero et al., 2009; Larcher and Scariot, 2009) or with pumice, coconut fibers, coconut "peat" and pine bark (Larcher and Scariot, 2009). Municipal solid waste compost was also used as fertilizer for potted geranium production (Ribeiro et al., 2000). Limited information is available about the use of rice hulls and anaerobic digestion residues of vegetal biomass in substrates for potted rose and geranium cultivation.

This study was aimed to evaluate the physical and chemical properties of 16 substrates containing fresh entire or ground rice hulls and fertilized or not with ADR and their effect on two different potted ornamentals.

## Material and Methods

### Substrates preparation

According to a 3 way factorial pattern, 16 substrates were prepared mixing limed white peat, rice hulls (RH) and anaerobic digestion residues (ADR) deriving from fruit distillery wastes. Four substrates, named U0-, U33-, U67- and U100-, were prepared by mixing peat with 0, 33, 67 and 100% respectively of unground rice hulls (URH) by volume. Four more substrates (U0+, U33+, U67+ and U100+) were prepared respecting the same mix proportions and fertilized with ADR (20% by final volume). Lastly, eight substrates, named G0-, G33-, G67-, G100-, G0+, G33+, G67+ and G100+, were prepared with the same percentage ratios but using 2mm ground rice hulls (GRH).

### Physical properties of substrates

Substrate bulk density (BD) was determined according to EN13040 (1999). Total pore space (TPS), water holding capacity (WHC) and air-filled porosity (AFP) were determined using an NCSU porometer according to Fonteno and Bilderback (1993). Each analysis was performed on 3 samples per substrate.

### **Chemical properties of substrates**

Substrate pH was measured in 1:5 substrate-water suspension according to EN 13037. The suspension used for pH determination was filtered and used for electrical conductivity (EC) determination according to EN 13038 methodology. Organic carbon (OC) and organic matter (OM) were determined after ashing 5 g of substrates using EN 13039. Dry matter (DM) of substrates was determined adopting EN 13040. Cation exchange capacity (CEC) was determined using BaCl<sub>2</sub>-triethanolamine (Lax et al., 1986). Total Kjeldahl nitrogen (TKN) was also measured. In order to obtain a measure of nutrients promptly available to plants water extraction with deionized water (EN 13652) was preferred to other official methods (e.g. EN 13651) even if it under-estimates total nutrient content. Hence nitrate-nitrogen (NO<sub>3</sub>-N), P, K, Ca, Mg and S were evaluated by using ionic chromatography (ICS-900, Dionex, Sunnyvale, CA, USA). Each analysis was performed on 3 samples per substrate.
#### Agronomic evaluation of substrates – Geranium experiment

Substrates were used to grow rooted cuttings of geranium (*Pelargonium hederipholium* 'Ville de Paris'). Plants were cultivated in a PE film greenhouse with openings in the roof and at the sides in which temperatures ranged from 14.3 to 26.3 °C on average. A minimum temperature of 13 °C was assured with a heating system. Pots used in the experiment had 14 cm diameter (1.2 L). Two rooted cuttings were transplanted per pot. Fertilization was done with one fertigation per week using a complete nutrient solution with 13-5-15 NPK ratio. Irrigation was different for each pot. Water volume applied per pot was recorded daily. After 35 days pot density was reduced from 24 to 12 pots m<sup>-2</sup>. The experiment was stopped at the 70<sup>th</sup> day of cultivation when the first plants reached marketable standard. Stem length, leaf, shoot and flower number, SPAD values (SPAD 502, Konica-Minolta, Japan), dry weight and dry matter of root, stem, leaf and flower and shoot:root ratio were determined. As rice seedlings in RH are considered weeds in substrates, they were also counted.

#### Agronomic evaluation of substrates – Rose experiment

The same PE film greenhouse was used for the rose (*Rosa* ×*hybrida* 'La Sevillana') cultivation experiment. Average temperatures ranged from 3.5 to 26.3 °C without supplementary heating. Ten cm square pots (1.1 L) were used. One rooted cutting was transplanted per pot. Substrates were previously fertilized with a slow release complete fertilizer (8-9 months) with 16-11-10 NPK ratio. Water volume per pot was recorded daily. On the 168<sup>th</sup> day of cultivation plant density was reduced from 50 to 25 pots m<sup>-2</sup>. The experiment was stopped after 210 days of cultivation when plants reached marketable standard. Plant height, total length and number of main shoots, secondary shoots number, leaf number, SPAD values, flower number, dry weight and dry matter of stem, leaf and flower were measured. Stem, leaf and flower repartition on aerial part biomass was determined. Rice seedlings were also counted.

#### Data statistical analysis

Data were analyzed by analysis of variance (ANOVA) and means separated according to Tukey's HSD test (P $\leq$ 0.05). Data on physical and chemical characterization were analyzed as a factorial experiment in a three way completely randomized design. Data

on both cultivation experiments were analyzed as a factorial experiment in three way randomized blocks with four repetitions. Values expressed as percentages were transformed prior to ANOVA analysis.

## **Results and Discussion**

#### Physical properties of substrates

All physical properties were significantly affected by the main factors (RH, ADR and grinding) and interactions "rate of GRH  $\times$  ADR treatment", "rate of GRH  $\times$  grinding" and "grinding  $\times$  ADR treatment" (Tab. 1). For this reason only interactions will be discussed. Substrates physical characteristics are reported in table. 2.

In ADR-unfertilized substrates, BD did not vary increasing RH content (0.211 g·cm<sup>-3</sup> on average), while in ADR-fertilized substrates BD decreased with progressive addition of RH, from 0.323 to 0.239 g·cm<sup>-3</sup>. BD did not differ increasing GRH in substrates (on average 0.239 g·cm<sup>-3</sup>) while it decreased by 41.4% increasing RH percentage in substrates. RH grinding and ADR addition to substrates increased their BD (Tab. 2). Even though all values were below 0.400 g·cm<sup>-3</sup>, the limit value for this parameter (Abad et al., 2011) treatment significantly influenced BD. The different observed trend may be partly due to issues concerning settling and packing of particles, especially when three components are mixed in a substrate rather than two (e.g. different size and shape of unground or ground RH and ADR compared to peat) (Buck and Evans, 2010).

Increasing RH content in substrates from 0 to 100%, TPS slightly decreased by 7.9% in substrates without ADR, but no differences were observed in ADR-fertilized substrates. There were only significant differences in substrates without RH, where ADR-unfertilized media presented TPS 6.0% higher than the corresponding ADR-fertilized substrates (Tab. 2). Again, increasing RH content, URH use did not affect TPS while GRH slightly reduced this parameter by 7.5% (Tab. 2). Both RH grinding and ADR addition reduced TPS of substrates. As seen for BD, also for TPS no differences were observed between substrates containing URH with ADR or GRH without ADR (Tab. 2). Bilderback et al. (2005) suggested an optimal TPS range between 50 and 85% and Arnold Bik (1983) and Boertje (1984) both recommended a minimum of 85% TPS. As TPS values ranged from 78.03% in G100+ to 90.61% in G0- all substrates were within the recommended range.

Bunt (1988) recommended an AFP of at least 10 to 20%. Jenkins and Jarrell (1989) and Handreck and Black (2002) proposed optimal ranges of 10 to 20% for AFP. De Boodt

and Verdonck (1972) suggested a 20-30% range for AFP. Bilderback et al. (2005) recommended AFP from 10 to 30%. AFP of studied substrates ranged from 9.71% in G33+ to 69.02% in U100- underlining wide differences between them. In particular, considering the interaction "rate of GRH × ADR treatment" it was observed that AFP increased by 187 and 205% in substrates without and with ADR respectively. Although AFP in substrates with ADR always resulted as lower than that in ADR-unfertilized, no significant difference was observed between treatments with the same RH percentage. Substrates with 67% RH and ADR-unfertilized, and substrates with 100% RH resulted in a too high AFP. RH in substrates increased AFP but it was observed that excessive values of some substrates are due to URH use. In fact, while with increasing GRH content in substrates values have increased AFP (from 16.6 to 64.9%), which was already higher than the suggested values with 33% URH. High AFP values of substrates with URH were confirmed by "grinding × ADR treatment" interaction in which ADR addition slightly decreased values but without significant differences (Tab. 2).

Optimal WHC ranges from 45% (Arnold Bik, 1983; Boertje, 1984) to 65% (Jenkins and Jarrell, 1989; Handreck and Black, 2002). Abad et al. (2001) proposed optimal WHC between 600 and 1000 ml/L. WHC was significantly reduced by increasing amount of RH, so that substrates with 100% RH presented too low values despite the ADR fertilization slightly improving this parameter in substrates with 33% RH or more. But as seen for increasing AFP values, decreasing WHC are also due to URH use, which reduced this parameter by 36.7, 90.6 and 167% in substrates with 33, 67 and100% RH respectively. In particular, substrates with 67 and 100% URH presented too low WHC. These results were reinforced by "grinding  $\times$  ADR treatment" that again showed lower WHC when URH was used than GRH (Tab. 2).

WHC and AFP showed different trends because WHC was calculated as the difference between TPS and AFP. High AFP and consequently low WHC of URH was already known (Kampf and Jung; 1991; Garcia-C. et al.; 2001; Evans and Gachukia, 2007), especially when rice hulls are added to substrates to improve AFP despite to WHC (Evans et al., 2011b). GRH physical properties closer to peat were also reported (Sambo et al., 2008). High AFP and low WHC of some substrates suggested more frequent water

applications (Gruda et al., 2000) but with smaller amounts (Benito et al., 2006).

#### Chemical properties of substrates

Chemical properties were significantly affected by the main factors (RH, ADR and grinding) and interactions "rate of GRH × ADR treatment", "rate of GRH × grinding" and "grinding × ADR treatment" (Tab. 3). Only interactions will be discussed, with the exception of Mg concentration for "rate of RH × ADR treatment", CEC, OM and OC and NO<sub>3</sub>-N for "rate of GRH × grinding" and DM for "grinding × ADR treatment" that resulted as not significant. Substrates chemical characteristics are reported in table 4.

Substrate pH ranged from 6.13 to 6.80 (Tab. 4). The peat used in the experiment had pH 6.13 due to lime application. ADR fertilization increased pH in all substrates, while RH grinding did not affect this parameter. RH content instead increased pH values in substrates with 33 and 67% RH compared to substrates with 0 and 100% RH. Pure ADR pH was higher than peat (7.27) and this may have raised the values in ADR-fertilized substrates. According to Abad et al. (2001), who proposed an optimal pH range between 5.3 and 6.5, substrates G33+, U67-, U67+, G67+, U100+ and G100+ presented values slightly higher than advisable, but respecting the wider range (5.5-7.0) proposed by Carlson and Rowey (1980).

Abad et al. (2001) suggested an EC level lower than 0.5 mS·cm<sup>-1</sup> while Handreck and Black (2002) and Zaccheo (2009) proposed a normal EC level range from 0.36 to 0.65 mS·cm<sup>-1</sup>. Substrates EC resulted as lower than the maximum level proposed by Abad et al. (2001) but it rose 4 fold with increasing RH content in ADR-unfertilized substrates, which in general appeared lower than advisable. Substrates were not previously fertilized and this may explain the low EC (Tab. 4). Increasing EC values due to higher rate of RH had already been observed (Gachukia and Evans, 2008; Zanin et al., 2011). As pure ADR presented 1.3 mS·cm<sup>-1</sup>, its use as fertilizer increased EC levels to normal range. RH grinding slightly increased EC, probably due to the release of a small amount of nutrient in the water extract (Bunt, 1988).

Increasing RH content in substrates decreased CEC by 78.4 and 70.0% in ADRunfertilized and ADR-fertilized substrates respectively, while RH grinding did not affect this parameter. Anyway ADR-fertilized substrates with GRH presented higher CEC values than ADR-unfertilized with GRH (17.9%), while substrates with URH had intermediate CEC levels (Tab. 4). High CEC values were reported by other authors (Bunt, 1988; Argo, 1998; Lemaire, 1999; Benito et al., 2006).

Because of the different moisture content of starting ingredients of substrates their DM increased with the increasing of RH content from 31.9 and 36.2 to 89.6 and 76.5% in ADR-unfertilized and ADR-fertilized. RH grinding lightly increased substrate DM (Tab. 4).

OM and OC in ADR-unfertilized substrates decreased by 11.5% while it increased by 16.1% in ADR-fertilized. ADR addition reduced substrates OM and OC but differences between substrates with the same RH percentage decreased (30.8, 24.2, 16.2 and 9.2% in substrates with 0, 33, 67 and 100% RH respectively). Interaction "grinding  $\times$  ADR treatment confirmed higher OM and OC in ADR-unfertilized substrates (Tab. 4). According to Abad et al. (2001) substrates without ADR presented optimal OM content (more than 80%). Lower values linked to ADR-fertilization are due to its lower content compared to peat. This is due to the industrial process that produced gas losing organic matter (Ward et al., 2008). Values for ADR-unfertilized substrates are similar to those reported by Zanin et al. (2011).

TKN content and Ca and S concentrations in the water extract showed the same behavior. Increasing RH content in growing media, values decreased by 42.7, 76.5 and 74.61 respectively in ADR-unfertilized substrates and by 49.9, 74.1 and 59.5% respectively in ADR-fertilized substrate. ADR use increased TKN, Ca and S contents in substrates by 2.8, 3.8 and 6.6 times compared to ADR-unfertilized growing media. Very few differences were observed increasing RH content in relation to grinding. ADR-fertilized substrates with URH presented higher nutrient levels, followed by those with GRH (Tab. 4). TKN of peat appeared lower than the range reported by Bunt (1988) for peat (1-2.5). Ca content is linked both to decreasing levels of CEC (Argo, 1998; Cattivello, 2009) due to the decreasing amount of peat (Cattivello, 2009) and also because it is a component of the peat liming ingredient. According to Pozzi and Valagussa (2009), Ca concentration appeared lower than advisable in ADR-unfertilized substrates and too high in U0+ and G0+. Despite ADR-fertilization S concentration appeared lower than advisable (Pozzi and Valagussa, 2009).

NO<sub>3</sub>-N concentration in the water extract of ADR-fertilized substrates decreased by 47.2% with increasing RH content. ADR-unfertilized substrates presented NO<sub>3</sub>-N values

close zero regardless of RH content or RH grinding. NO<sub>3</sub>-N concentration in ADR-fertilized substrates with URH was 33.9% higher than that with GRH (Tab. 4). Values of ADR-fertilized substrates were within the normal range (11-23 mg/L) proposed by Pozzi and Valagussa (2009).

Regardless of ADR-fertilization or RH grinding, P and K concentrations in water extract rose by 12.2 and 3.4 times respectively with increasing RH content. ADR addition to substrates improved P and K concentrations. RH grinding produced a slight reduction in P concentration in ADR-fertilized substrates but not in ADR-unfertilized, while it slightly increased K (Tab. 4). It is already known that RH presents higher levels of K (Cadell, 1988; Cattivello, 2009; Zanin et al., 2011) and P (Evans et al., 2011a) than peat. While P concentration values appeared lower than recommended, K concentration appeared too high (Pozzi and Valagussa, 2009).

Mg concentrations resulted as similar in ADR-unfertilized substrates. RH grinding slightly reduced Mg concentration in substrates with RH but significant differences were detected only in substrates with 67% RH. Compared to ADR-unfertilized substrates, ADR addition increased Mg concentration by 81.0% in substrates with GRH and by 213% in those with URH (Tab. 4). Despite ADR addition Mg values in water extract appeared lower than advisable (Pozzi and Valagussa, 2009).

#### Agronomic evaluation of substrates – Geranium experiment

Among the main factors, RH content in substrates affected all the considered parameters, while some of them were not affected by ADR fertilization or by RH grinding. Some first level interactions also affected the considered parameters. Significance of main effects and interactions are reported in table 5. Results significant at  $P \le 0.01$  are presented below.

Plant stem length (Fig. 1) decreased raising RH content in substrates. Geranium cultivated with 67 and 100% RH resulted as 12.9 and 22.3% shorter respectively than those grown with 0% RH. Both ADR fertilization and use of GRH permitted values 7.6 and 3.8% higher respectively than substrates without ADR or with URH. No significant differences were observed on SPAD values increasing RH content up to 67%. Plants grown in 100% RH presented values 6.1% lower than 0% RH (Fig. 2). ADR fertilization reduced (-4.4%) plants SPAD values (Fig. 2). Considering the main factors, increasing RH content in the

substrate reduced leaf number (-59.2%) but use of ADR and GRH generally counteracted this negative effect, increasing leaf number by 8.7 and 9.7% respectively (Fig. 3). Leaf number was significantly affected by the interaction "rate of RH × ADR treatment". Although plants grown in substrates with 33% RH had fewer leaves than those in 0% RH, no significant differences were observed. Higher RH further reduced leaf number but ADR fertilization limited decreasing values in substrates with 67% RH (Fig. 4). Shoot number (Fig. 5) was reduced increasing RH content by 99.0%, while plants grown on ADRfertilized substrates presented 12.0% higher shoot number compared to those grown without ADR. Considering RH grinding, values were 12.2% higher in substrates with GRH than media with URH. Increasing RH content also reduced flower number (-43.4%) from 5.18 to 2.96, but considering the interaction "RH  $\times$  ADR" ADR addition increased flower number by 41.2% in substrates with 67% RH to values comparable to plants grown with 0 and 33% RH (Fig. 6). Root dry weight increased by 27.6% with RH percentage, while it was reduced by ADR addition (-10.4%) (Tab. 6). Stem and leaf dry weight were reduced (-54.1 and -46.7% respectively) by increasing RH content but stimulated using ADR (+15.0 and +14.3%) and GRH (+16.2 and 12.3%) (Tab. 6). ADR fertilization increased leaf number in substrates with 33% RH or more, but only ADR-fertilized substrates with 33% RH presented values significantly higher than the corresponding substrates not fertilized with ADR (Fig. 7). Increasing RH percentage also reduced flower dry weight (-49.9%) (Tab. 6). Fertilization with ADR related to increasing RH showed similar behavior to that observed for flower number but in this case not significant differences were observed between substrates with the same RH content (Fig. 8). Although significant differences were observed on leaf dry matter with increasing RH content in substrates, the values of those with 0% RH resulted as not significantly different from the others (Tab. 6). Leaf dry matter was reduced (-4.2%) by ADR fertilization. Shoot:root ratio (Fig. 9) was negatively affected by increasing RH content (-28.8%) in the substrates but it was increased by ADR fertilization (+7.6%) and by GRH use (3.8%) (Fig. 9). Considering biomass partitioning, stem and leaf percentage decreased with 67 and 100% RH. Flower percentage decreased starting with plants grown in substrates with 33% RH. Root percentage instead increased with RH content in the studied media (Fig. 10). ADR fertilization negatively affected root and flower percentage (-19.2 and -5.8% respectively) but slightly increased stem and leaf percentage (+4.9 and +3.0%) (Fig. 11). Stem percentage resulted as 5.2% higher when GRH was used compared to URH, while there was no significant difference for root, leaf and flower percentages (Fig. 12).

Considering the main factors, substrates with 100% RH received more water than the other substrates (on average 42.5%), increasing from 2036 to 2947 ml/pot and GRH permitted 8.7% water to be saved than URH (Fig. 13). ADR fertilization reduced water volumes applied to plants in substrates with 67 and 100% RH even though they resulted as not significantly different from the corresponding unfertilized substrates (Fig. 14). RH grinding permitted water to be saved in substrates with 67 and 100% RH. In particular water saved using GRH in substrates with pure RH was 438 ml/pot (-13.8%) (Fig. 15). RH grinding did not affect water volume in substrates with ADR, while it resulted as determinant to save water in substrates without ADR (Fig. 16).

Geranium aerial parameters were all reduced by increasing RH content in the substrate. Similar behavior was observed by Evans and Gachukia (2004), who reported a reduction in stem dry weight of geranium even though no significant differences were found between substrates in that experiment. This reduction also involved some other annual ornamentals. A growth reduction in pepper seedlings was reported when plants were grown in substrates containing fresh rice hulls (Lee et al., 2000a, 2000b) or increasing rate of rice hulls (Zanin et al., 2011). Oh et al. (2007) observed a reduction of some biometric parameters in kalanchoe linked to physical properties of different substrates when water supply was reduced. Hence according to Zanin et al. (2011), decreasing values obtained for geranium aerial parameters are probably linked to inferior physical properties of substrates that in some cases showed too high AFP to the detriment of WHC. Root parameters values increased with RH content in the substrates. Taiz and Zeiger (1998) reported that plants with water stress stimulate root production to find moisture. In this experiment pots were watered daily as needed but this does not exclude that substrates with higher AFP and lower WHC lost water faster than the others, inducing higher root production. Use of GRH with respect to URH improved the physical properties of substrates and this also permitted the production of plants with better characteristics. The addition of ADR slightly improved physical properties and provided a higher nutrient level. This may explain why ADR addition generally improved values of the considered parameters. Other authors using

decomposed biomasses (composted agro-wastes, manure or sewage sludge) observed higher parameters in ornamental plants and attributed this positive effect to higher nutrient content (Atiyeh et al., 2001; Garcia-Gomez, 2002; Ostos et al., 2008; Herrera et al., 2008). D'Angelo et al. (1993) concluded an experiment on ornamental plants reporting that physical analyses were more useful than chemical ones in explaining responses to cultivation substrates. In this experiment too, the better chemical profile of some substrates appeared insufficient to equilibrate poorer physical properties. A similar explanation was given by Zanin et al. (2011).

Rice seedlings represent a negative aspect in ornamental plants so they should be removed. While they were absent in peat substrates, with increasing RH content rice seedling number reached a maximum in 67% RH but not significant differences were observed between RH rates, and URH use presented a rice seedling number 26 times higher than GRH (Fig. 17). Considering the "rate of RH × RH grinding" interaction, excluding peat substrates, while the substrates with GRH had less than one seedling per pot those with URH showed markedly higher values (more than 13 rice seedlings). According to Evans and Gachukia (2004), rice seedlings germinated from rice seeds remaining in the hulls after milling. As the RH used in this experiment had not undergone any treatment, this could explain the high rice seedlings number in substrates with URH and confirms the suggestion by some authors that parboiled rice hulls should be preferred because this process destroys vital seeds (Einert, 1972; Evans and Gachukia, 2004). Although RH grinding has not assured total rice seedling destruction, the process reduced vital seeds to less than 1 seed per pot.

#### Agronomic evaluation of substrates – Rose experiment

As regards main factors, rate of RH and RH grinding affected some parameters while, with the exception of water volume and rice seedling number, no parameter resulted as being affected by ADR fertilization. First level interaction also affected some of the considered parameters. Significance of main effects and interactions are reported in table 7 and again results significant at  $P \le 0.01$  are presented below.

Increasing RH content plant height and total shoots length was reduced by 22.6% but was 11.6% higher in ADR-fertilized substrates (Fig. 19). Roses grown in substrates with URH presented the same behavior as that described for the main effect of RH rate in

the substrate, with a height reduction of 36.9% against a not significant reduction of 7.6% observed in plants grown in substrates with GRH (Fig. 20). Total shoot length (Fig. 21), secondary shoots number (Fig. 22), leaf number (Fig. 23) and flower number were all negatively affected by increasing rate of RH in the substrates, with a reduction in values of 27.3, 76.1, 32.9 and 37.53% respectively. Observing the interaction "rate of RH × ADR treatment" ADR fertilization slightly reduced flower number in substrates with 0 and 33% RH and sustained this parameter in substrate with 67 and 100% RH but without significant differences between substrates with the same RH content (Fig. 24). With increasing RH rate in substrates stem, leaf and flower dry weight decreased by 39.2, 36.6 and 36.1% (Tab. 7). Stem and leaf dry weight were 15.7 and 13.1% higher when plants were cultivated in substrates with GRH compared to those with URH (Tab. 7). ADR fertilization sustained stem dry weight in substrates with 67% or more RH and slightly reduced this parameter on plants grown with 0 and 33% RH, but not significant differences were observed between substrates with the same RH content (Fig. 25). Stem dry weight was strongly reduced increasing URH content in the substrates (-53.7%). The use of GRH limited this reduction to -24.4% (Fig. 26). No differences were observed in stem dry matter of plants grown in 0 and 33% RH. Further RH increase raised this value by 12.5% (Tab. 7). In the biomass partitioning stem percentage was limited by increasing RH content (data not reported) and positively affected by GRH use (+3.6%) (Fig. 27).

All main factors and interactions showed a significant effect on water use in rose cultivation. Increasing water volumes (from 978 to 2940 ml/pot) were applied increasing RH content in substrates. The use of ADR and GRH saved 19.0 and 20.3% of water respectively (data not reported). ADR addition to substrates reduced water requirements in substrates with 67 and 100% RH (-22.1 and -23.7%), although the difference was significant only for substrates with 100% RH (Fig. 28). A similar effect was observed increasing RH content using ground or not ground. GRH reduced water consumption starting from substrates with 33% RH but the effect only resulted as significant with 100% RH (Fig. 29). As for geranium, substrates with URH without ADR were those with higher water consumption (2274 ml/pot). ADR-fertilized substrates with GRH saved 34.0% of water on average (Fig. 30).

RH ratio in the substrate also appeared to be the limiting factor in rose cultivation,

but this ornamental better tolerated the rice byproduct than geranium. As discussed for geranium, the reduction of the described rose parameters is probably mainly due to lower physical properties of substrates with 67% or more RH. ADR addition to substrates did not affect any parameter, confirming that nutritional support is less important than physical properties (D'angelo et al., 1993; Zanin et al., 2011). RH grinding improved only a few parameters but it was key to saving water with high RH rate in the substrates. Increasing RH content in the substrate corresponded to higher water volumes that resulted as effective only up to 33% RH. With more than 33% RH a partial nutrient loss as an effect of higher water volumes applied to those substrates may be added to lower physical properties.

Considering main factors, more rice seedlings were found in rose pots containing substrates with 67% RH (8.4 plants/pot). These decreased in substrates with 100 and 33% RH and were obviously absent in peat substrate. Substrates with ADR had a higher number of rice seedlings compared to those without this byproduct and substrates with URH had 14.7 higher levels of rice seedlings than those with GRH (Fig. 31). Among interactions, the most interesting is "rate of RH × RH grinding". Excluding substrates without RH, with increasing RH content, those with URH were respectively 13.8, 16.1 and 14.2 times higher than the corresponding ones with GRH (Fig. 32).

## Conclusions

RH rate in substrates modified their physical properties, strongly increasing AFP and consequently reducing WHC sometimes to levels outside the optimal range (e.g. 100% RH). GRH use permitted better values to be obtained, generally within the proposed optimal range. ADR fertilization should not have modified physical properties but its addition showed a positive effect, similar to but smaller than GRH use.

The studied factors, especially RH and ADR addition, also modified the chemical characteristics. Increasing RH content implied a rise of DM, P and K contents in the water extract values but also decreases of CEC, nitrogen percentage, NO<sub>3</sub>-N Ca and S. These different trends may cause nutritional imbalance. This problem was solved with ADR addition that increased all nutrient levels in the water extract. RH grinding slightly modified some chemical characteristics.

In the cultivation experiments geranium resulted as negatively affected by increasing rate of RH, which involved all parameters considered probably because of worsening of substrate physical properties. Both ADR fertilization of substrates and GRH use instead of URH improved many parameters. In general it is possible to partly substitute peat with 33% RH but to avoid producing smaller plants RH should be ground and the substrate should be fertilized with ADR.

Rose cultivation data confirmed the negative effect of RH in the substrate but in general a partial substitution of peat with 33% RH resulted as being possible independently of ADR fertilization or RH grinding. ADR did not affect plant growth while GRH use is preferred to improve some parameters.

The economic and environmental benefits achievable also present two negative aspects regarding higher water consumption and rice seedling presence. These problems may both be partly solved by using GRH and ADR in the substrates, as also suggested for growing potted geranium and roses.

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## Tables

Table 1. Main effect "rate of RH" (A), "ADR treatment" (B) and "RH grinding (C)" and interaction on bulk density (BD) total pore space (TPS), air-filled porosity (AFP) and water holding capacity (WHC) (values indicate % by volume).

	А	В	С	$\mathbf{A} \times \mathbf{B}$	$A \times C$	$\mathbf{C} \times \mathbf{B}$	$A \times B \times B$
BD (g·cm <sup>-3</sup> )	***	***	***	***	***	***	***
TPS (%)	***	***	***	***	***	***	**
AFP (%)	***	***	***	*	***	**	n.s.
WHC (%)	***	***	***	***	***	***	n.s.

Values with the same letter are not different according to Tukey's HDR test (P $\leq$ 0.05). \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant.

Table 2. Effects of interaction "rate of RH (A) × ADR treatment (B)", "rate of RH (A) × RH grinding (C)" and "RH grinding (C) × ADR treatment (B)" on bulk density (g·cm<sup>-3</sup>) (BD) total pore space (TPS), air-filled porosity (AFP) and water holding capacity (WHC) (values indicate % by volume). 0, 33, 67 and 100 is RH percentage in substrates; - = without ADR; + = with ADR; U = unground RH; G = ground RH. Sig. =Significance.

	rate of GRH (A) $\times$ ADR treatment (B)											
	0-	33-	67-	100-	0+	33+	67+	100+	$\mathbf{A} \times \mathbf{B}$			
BD	0.219 b	0.211 b	0.200 b	0.215 b	0.323 a	0.309 a	0.275 ab	0.239 ab	***			
TPS	90.47 a	86.32 ab	85.58 ab	83.32 b	85.01 b	82.69 b	81.70 b	81.50 b	***			
AFP	16.00 ab	26.59 ab	39.01 ab	45.94 a	12.92 b	19.10 ab	29.83 ab	39.45 ab	*			
WHC	74.47	59.73	46.57	37.38	72.09	63.60	51.87	42.05	***			
	а	abcd	bcd	d	ab	abc	abcd	cd				
	rate of GRH (A) $\times$ GRH grinding (C)											
	0U	33U	67U	100U	0G	33G	67G	100G	$\mathbf{A} \times \mathbf{C}$			
BD	0.266 ab	0.249 ab	0.204 bc	0.156 c	0.276 a	0.271 ab	0.270 ab	0.299 ab	***			
TPS	87.62 a	86.27 ab	86.83 ab	86.54 ab	87.86 a	82.75 bc	80.45 c	78.28 c	***			
AFP	16.56 de	34.17 c	52.94 b	64.90 a	12.35 e	11.52 e	15.89 de	20.49 d	***			
WHC	71.05 a	52.10 c	33.88 d	21.64 e	75.51 a	71.23 a	64.56 b	57.78 c	***			
			GRH g	rinding (C) $\times$	ADR treatm	nent (B)			Sig.			
	U	J-	U	+	(	3-	G	i+	$\mathbf{C} \times \mathbf{B}$			
BD	0.1	71 c	0.26	7 ab	0.2	52 b	0.30	06 a	***			
TPS	88.9	90 a	84.7	73 b	83.	95 b	80.72 c		***			
AFP	46.0	63 a	37.0	56 a	17.	14 b	12.99 b		***			
WHC	42.2	27 b	47.0	)7 b	66.	81 a	67.2	73 a	***			

Values with the same letter are not different according to Tukey's HDR test (P $\leq$ 0.05). \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant.

	А	В	С	$\mathbf{A} \times \mathbf{B}$	$A \times C$	$\mathbf{C} \times \mathbf{B}$	$A \times B \times B$
рН	***	***	n.s.	***	***	**	***
EC (mS·cm <sup>-1</sup> )	***	***	***	***	***	***	***
CEC (meq·100g)	***	***	n.s.	***	n.s.	***	**
Dry matter (%)	***	***	***	***	***	n.s.	***
OM (%)	***	***	***	***	n.s.	***	***
CO (%)	***	***	***	***	n.s.	***	***
TKN (%)	***	***	***	***	***	***	***
NO3-N (mg·L-1)	***	***	***	***	n.s.	***	***
P (mg·L-1)	***	***	***	***	***	***	***
K (mg·L-1)	***	***	***	***	***	***	***
Ca (mg·L-1)	***	***	***	***	***	***	**
Mg (mg·L-1)	n.s.	***	***	n.s.	*	***	**
S (mg·L-1)	***	***	n.s.	***	***	***	***

Table 3. Main effect "rate of RH" (A), "ADR treatment" (B) and "RH grinding (C)" and interactions on chemical properties of substrates.

Values with the same letter are not different according to Tukey's HDR test (P $\leq$ 0.05). \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant.

Table 4. Effects of interaction "rate of RH (A) × ADR treatment (B)", "rate of RH (A) × RH grinding (C)" and "RH grinding (C) × ADR treatment (B)" on chemical properties of substrates. 0, 33, 67 and 100 are the RH percentage in substrates; - or + = without or with ADR; U or G = unground or ground RH.

	pН	EC	CEC	DM	ОМ	СО	TKN	NO3-N	Р	K	Ca	Mg	S
0-	6.13 de	0.063 d	190.9 a	31.87 f	93.60 a	54.29 a	0.456 de	0.47 d	0.00 c	0.98 d	6.17 bc	0.90	2.03 cd
33-	6.28 cd	0.102 cd	144.2 b	46.00 de	88.12 b	51.11 ab	0.380 e	0.01 d	1.51 b	15.93 cd	6.21 bc	0.61	1.18 de
67-	6.55 ab	0.198 bc	91.1 cd	71.35 bc	84.05 bc	48.75 b	0.355 e	0.02 d	3.73 a	41.34 bc	3.20 c	0.59	0.30 de
100-	6.00 e	0.258 b	41.2 e	89.59 a	82.82 c	48.04 b	0.261 e	0.48 d	4.56 a	65.39 ab	1.45 c	0.97	0.51 e
0+	6.43 bc	0.408 a	146.2 b	36.17 ef	64.75 f	37.56 e	1.340 a	20.95 a	0.92 bc	44.23 b	26.33 a	1.90	8.83 a
33+	6.63 ab	0.446 a	118.3 bc	47.58 d	66.81 ef	38.75 de	1.130 ab	19.37 ab	1.28 bc	65.94 ab	20.62 a	2.01	8.26 a
67+	6.73 a	0.387 a	64.6 de	60.91 c	70.43 de	40.85 cd	0.874 bc	12.00 bc	0.78 bc	72.55 a	11.56 b	1.80	5.82 b
100 +	6.70 a	0.383 a	43.8 e	76.54 b	75.16 d	43.60 c	0.671 cd	11.06 c	4.47 a	90.47 a	6.81 bc	1.87	3.57 c
A×B	***	***	**	***	***	***	***	***	***	***	***	ns	***
0U	6.30 bc	0.198 b	170	33.80 e	78.89	45.76	0.922 a	9.21	0.45 d	23.03 b	16.94 a	1.38 ab	4.62 ab
33U	6.42 bc	0.278 ab	134	45.02 de	75.06	43.54	0.861 ab	11.42	2.28 c	39.20 b	16.41 ab	1.46 ab	4.69 ab
67U	6.75 a	0.307 ab	70	58.87 c	75.19	43.61	0.790 ab	9.92	2.78 bc	52.49 b	11.52 ab	1.72 a	3.78 ab
100U	6.25 c	0.258 ab	42	79.07 ab	78.42	45.49	0.591 abc	7.94	4.06 ab	57.47 ab	6.24 ab	1.78 a	2.01 b
0G	6.27 c	0.273 ab	157	34.24 e	79.46	46.09	0.874 ab	12.20	0.47 d	22.17 b	15.57 ab	1.42 ab	6.24 a
33G	6.50 abc	0.269 ab	129	48.56 cd	79.86	46.32	0.650 abc	7.95	0.51 d	42.66 b	10.42 ab	1.16 ab	4.76 ab
67G	6.53 ab	0.278 ab	86	73.39 b	79.30	46.00	0.438 bc	2.10	1.72 cd	61.40 ab	3.24 ab	0.68 b	2.34 b
100G	6.45 bc	0.383 a	43	87.06 a	79.56	46.15	0.341 c	3.60	4.96 a	98.40 a	2.02 b	1.06 ab	2.08 b
A×C	**	***	ns	***	ns	ns	***	ns	***	***	***	*	***
U-	6.27 b	0.114 b	105.8 a	58.46	87.51 a	50.76 a	0.399 c	0.17 c	2.86	20.76 c	4.65 c	0.77 c	0.79 b
U+	6.59 a	0.407 a	102.4 ab	49.92	66.27 b	38.44 c	1.183 a	19.08 a	1.93	65.33 ab	20.90 a	2.40 a	6.75 a
G-	6.22 b	0.197 b	111.9 ab	60.94	86.79 a	50.34 a	0.326 c	0.32 c	2.04	41.05 bc	3.87 c	0.77 c	1.22 b
G+	6.66 a	0.405 a	95.2 b	60.68	72.31 b	41.94 b	0.825 b	12.60 b	1.79	71.26 a	11.75 b	1.39 b	6.49 a
C×B	***	***	***	ns	***	***	***	***	ns	***	***	***	***

Values with the same letter are not different according to Tukey's HDR test ( $P \le 0.05$ ). \*\*\*, \*\* and \* = significant at  $P \le 0.001$ , 0.01 and 0.05, respectively. n.s. =

not significant.

Geranium	Α	В	С	$\mathbf{A} \times \mathbf{B}$	A × C	C × B
Stem length (cm)	***	***	**	n.s.	n.s.	n.s.
SPAD	***	***	n.s.	*	n.s.	*
Leaf (n°)	***	***	***	**	n.s.	n.s.
Shoot (n°)	***	***	***	n.s.	n.s.	n.s.
Flower (n°)	***	n.s.	n.s.	***	n.s.	n.s.
Root dry weight (g)	***	**	n.s.	n.s.	n.s.	*
Stem dry weight (g)	***	***	***	*	n.s.	n.s.
Leaf dry weight (g)	***	***	***	n.s.	***	*
Flower dry weight (g)	***	n.s.	n.s.	***	*	n.s.
Root dry matter (%)	*	*	n.s.	n.s.	*	n.s.
Stem dry matter (%)	*	n.s.	n.s.	n.s.	n.s.	n.s.
Leaf dry matter (%)	***	**	n.s.	n.s.	n.s.	n.s.
Flower dry matter (%)	*	*	n.s.	n.s.	n.s.	n.s.
Shoot:root ratio	***	***	**	*	n.s.	*
Root (%)	*	*	n.s.	n.s.	*	n.s.
Stem (%)	***	**	**	*	n.s.	n.s.
Leaf (%)	***	***	n.s.	n.s.	n.s.	n.s.
Flower (%)	*	*	n.s.	n.s.	n.s.	n.s.
Water volume (ml/pot)	***	*	**	**	***	**
Rice seedlings (n°)	***	n.s.	***	*	***	n.s.

Table 5. Main effect "rate of RH" (A), "ADR treatment" (B) and "RH grinding (C)" and interactions on geranium parameters.

Values with the same letter are not different according to Tukey's HDR test (P $\leq$ 0.05). \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant.

	RH content			ADR fer	tilization	RH grinding		
	0	33	67	100	-ADR	+ ADR	URH	GRH
Root DW (g)	0.75 b	0.85 b	0.87 ab	0.96 a	0.90 a	0.81 b	0.84	0.87
Stem DW (g)	3.25 a	2.81 b	2.10 c	1.49 d	2.24 b	2.58 a	2.23 b	2.59 a
Leaf DW (g)	6.25 a	5.74 b	4.49 c	3.33 d	4.62 b	5.28 a	4.67 b	5.24 a
Flower DW (g)	1.37 a	1.11 b	0.96 b	0.69 c	1.02	1.05	1.03	1.04
Root DM (%)	12.88 a	11.73 ab	11.07 b	11.64 ab	11.43 b	12.22 a	12.10	11.56
Stem DM (%)	13.48 ab	13.15 b	13.94 a	14.01 a	13.83	13.46	13.68	13.61
Leaf DM (%)	9.56 ab	9.27 b	9.91 a	10.02	9.90 a	9.48 b	9.69	9.69
Flower DM (%)	12.24 b	12.31 b	13.07 ab	13.53 a	13.16 a	12.42 b	12.90	12.68

Table 6. Effects of rate of RH (A), ADR treatment (B) and RH grinding (C) on geranium root, stem, leaf and flower dry weight (DW) and dry matter (DM). 0, 33, 67 and 100 are the RH percentage in substrates; - = without ADR; + = with ADR; U = unground RH; G = ground RH. Sig. =Significance.

Values with the same letter are not different according to Tukey's HDS test ( $P \le 0.05$ ).

Rose	Α	В	С	$\mathbf{A} \times \mathbf{B}$	$\mathbf{A} \times \mathbf{C}$	C × B
Plants height (cm)	***	n.s.	**	n.s.	**	n.s.
Total shoots length (cm)	***	n.s.	n.s.	n.s.	*	n.s.
SPAD	n.s.	n.s.	n.s.	*	n.s.	n.s.
Main shoots (n°)	n.s.	n.s.	*	n.s.	n.s.	n.s.
Secondary shoots (n°)	***	n.s.	n.s.	n.s.	n.s.	n.s.
Leaf (n°)	***	n.s.	n.s.	n.s.	n.s.	n.s.
Flower (n°)	***	n.s.	n.s.	**	n.s.	n.s.
Stem dry weight (g)	***	n.s.	**	**	**	n.s.
Leaf dry weight (g)	***	n.s.	***	*	*	n.s.
Flower dry weight (g)	***	n.s.	*	*	*	n.s.
Stem dry matter (%)	***	n.s.	n.s.	n.s.	n.s.	n.s.
Leaf dry matter (%)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Flower dry matter (%)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Stem (%)	*	n.s.	**	n.s.	n.s.	n.s.
Leaf (%)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Flower (%)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Water volume (ml/pot)	***	***	***	***	***	***
Rice seedlings (n°)	***	***	***	**	***	***

Table 7. Main effect "rate of RH" (A), "ADR treatment" (B) and "RH grinding (C)" and interactions on rose parameters.

Values with the same letter are not different according to Tukey's HDR test (P $\leq 0.05$ ). \*\*\*, \*\* and \* = significant at P  $\leq 0.001$ , 0.01 and 0.05, respectively. n.s. = not significant.

	RH content			ADR fer	tilization	RH grinding		
	0	33	67	100	-ADR	+ ADR	URH	GRH
Stem DW (g)	3.63 a	3.19 a	2.60 b	2.20 b	2.90	2.91	2.69 b	3.12 a
Leaf DW (g)	7.67 a	6.87 a	5.84 b	4.86 c	6.33	6.29	5.93 b	6.70 a
Flower DW (g)	2.18 a	1.90 ab	1.68 bc	1.39 c	1.73	1.85	1.71 b	1.87 a
Stem DM (%)	32.40 a	32.57 a	35.51 b	36.64 b	34.76	33.80	34.50	34.06
Leaf DM (%)	30.49	29.97	31.93	32.17	31.52	30.76	31.22	31.06
Flower DM (%)	20.49	20.14	21.12	21.34	20.90	20.64	20.53	21.01

Table 8. Effects of rate of RH (A), ADR treatment (B) and RH grinding (C) on rose stem, leaf and flower dry weight (DW) and dry matter (DM). 0, 33, 67 and 100 are the RH percentage in substrates; - = without ADR; + = with ADR; U = unground RH; G = ground RH. Sig. =Significance.

Values with the same letter are not different according to Tukey's HDS test ( $P \le 0.05$ ).

# **Figures**



Figure 1. Effect of rate of rice hulls (RH) content, addition (+) or not (-) of anaerobic digestion residues (ADR) and RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on geranium stem length. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 2. Effect of rate of rice hulls (RH) content, addition (+) or not (-) of anaerobic digestion residues (ADR) on geranium SPAD value. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 3. Effect of rate of rice hulls (RH) content, addition (+) or not (-) of anaerobic digestion residues (ADR) and RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on geranium leaf number. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 4. Effect of interaction "RH × ADR" on geranium leaf number. (RH = relative rate of rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 5. Effect of rate of rice hulls (RH) content, addition (+) or not (-) of anaerobic digestion residues (ADR) and RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on geranium shoot number. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 6. Effect of interaction "RH × ADR" on geranium flower number. (RH = relative rate of rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 7. Effect of interaction "RH × RH grinding" on geranium leaf dry weight. (RH = relative rate of rice hulls; UGR = unground rice hulls; GRH ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 8. Effect of interaction "RH × ADR" on geranium flower dry weight. (RH = relative rate of rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 9. Effect of rate of rice hulls (RH) content, addition (+) or not (-) of anaerobic digestion residues (ADR) and RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on geranium shoot:root ratio. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 10. Effect of rate of rice hulls (RH) content on root, steam, leaf and flower repartition of geranium. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 11. Effect of rate of anaerobic digestion residues (ADR) on root, steam, leaf and flower repartition of geranium. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 12. Effect of rice hulls (RH) grinding on root, steam, leaf and flower repartition of geranium. (UGR = unground rice hulls; GRH = ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 13. Effect of rate of rice hulls (RH) content and RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on water volume required by geranium during cultivation. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 14. Effect of interaction "RH × ADR" on water volume required by geranium during cultivation. (RH = relative rate of rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 15. Effect of interaction "RH × RH grinding" on water volume required by geranium during cultivation. (RH = relative rate of rice hulls; UGR = unground rice hulls; GRH ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 16. Effect of interaction "RH grinding × ADR" on water volume required by geranium during cultivation. (UGR = unground rice hulls; GRH ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 17. Effect of rate of rice hulls (RH) content and RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on rice seedlings number in geranium pots. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 18. Effect of interaction "RH × RH grinding" on rice seedlings number in geranium pots. (RH = relative rate of rice hulls; UGR = unground rice hulls; GRH ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 19. Effect of rate of rice hulls (RH) content and RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on rose height. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 20. Effect of interaction "RH × RH grinding" on rose height. (RH = relative rate of rice hulls; UGR = unground rice hulls; GRH ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.


Figure 21. Effect of rate of rice hulls (RH) content on rose total shoots length. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 22. Effect of rate of rice hulls (RH) content on rose secondary shoots number. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 23. Effect of rate of rice hulls (RH) content on rose leaf number. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 24. Effect of interaction "RH × ADR" on rose flower number. (RH = relative rate of rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 25. Effect of interaction "RH × ADR" on rose stem dry weight. (RH = relative rate of rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 26. Effect of interaction "RH × RH grinding" on rose stem dry weight. (RH = relative rate of rice hulls; UGR = unground rice hulls; GRH ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 27. Effect RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on rose steam, leaf and flower repartition. Values of histogram with the same letter are not different according to Tukey's HDS test (P≤0.05).



Figure 28. Effect of interaction "RH × ADR" on water volume required by rose during cultivation. (RH = relative rate of rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 29. Effect of interaction "RH × RH grinding" on water volume required by rose during cultivation. (RH = relative rate of rice hulls; UGR = unground rice hulls; GRH ground rice hulls). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 30. Effect of interaction "RH grinding× ADR" on water volume required by rose during cultivation. (UGR = unground rice hulls; GRH ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 31. Effect of rate of rice hulls (RH) content, addition (+) or not (-) of anaerobic digestion residues (ADR) and RH grinding (unground rice hulls [URH] or ground rice hulls [GRH]) on rice seedlings number on rose pots. Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.



Figure 32. Effect of interaction "RH grinding× ADR" on rice seedlings number on rose pots. (UGR = unground rice hulls; GRH ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to Tukey's HDS test (P≤0.05). Vertical bars represent ± SE of means.

**Chapter VI** 

Rooting and cultivation of miniature rose (*Rosa ×hybrida* 'Tilt Meillandina') in rice hull-based substrates fertilized with anaerobic digestion residues

## Abstract

Rooting and potting media are one of the factors that mostly influence propagation and production of high quality plants. Substrates are generally composed of peat mixed with other inorganic components. In order to limit peat use and exploit two agro-industrial byproducts, some substrates were tested as rooting and growing media for 'Tilt Meillandina' miniature rose production. Four substrates were prepared mixing peat with ground rice hulls (GRH) at different percentages (0, 33, 67 and 100%). Four more substrates were prepared with these peat-rice hulls mixes fertilized with anaerobic digestion residues (ADR) deriving from vegetal biomasses (20% by volume). A high percentage of GRH in the substrate negatively affected rooting percentage, this resulted in a considerable reduction percentage of marketable pots in 100% GRH substrates. ADR addition reduced percentage of rooting at low percentages of GRH but did not affect the percentage of marketable pots. Plant growth was often negatively affected by 67% or higher of GRH but addition of ADR to these substrates improved plant growth, which was often comparable to that of the peat control. In conclusion, total substitution of peat with GRH and ADR appeared not to be feasible but a partial replacement with a high percentage of GRH seemed possible if fertilization with ADR is adopted.

### Introduction

Peat is characterized by good physical and chemical properties, such as high porosity, high water-holding capacity, and relatively high cation-exchange capacity. For this reason peat is the most important ingredient of substrates for containerized plant production (Li et al., 2009). The environmental concerns associated with peat exploitation have led to a reduction of its extraction in many areas (Barkham, 1993; Robertson, 1993; Gallagher, 2008; Shober et al., 2010). As a consequence, the increasing price of substrates (Ostos et al., 2006) encouraged researchers to investigate the suitability of alternative materials to peat in a sustainable approach (Ribeiro et al., 2007; Marianthi, 2006; Iglesias-Díaz et al., 2009). No ideal substrate exists so, in order to achieve desirable physical and chemical properties, many materials are often mixed together (Bunt, 1971; Fonteno, 1993; Bachman and Metzger, 2007; Hartmann et al., 2002).

Rooting substrates should present adequate water and air space balance to assure

water for cuttings uptake and supply sufficient aeration for adventitious rooting (Hartmann et al., 2002). Different experiments outlined how baldcypress rooted better in the wettest substrates (Copes and Randal; 1993; King et al., 2011) while some other ornamental woody species performed better in substrates with more favorable air-filled porosities (Chong and Dale, 2004). In rooting experiments different materials such as sawdust, sand, gravel (Ofori et al., 1996; Mésen et al., 2007; Tchoundjeu et al., 2002; Atangana et al., 2006), fine bark, coconut coir, perlite (Stoven and Kooima, 1999), pumice (Talaie and Nejatie, 1999) and grape pomace (Chong and Dale, 2004) were used with not always encouraging results.

In the cultivation stage many experiments proved that it is possible to partially substitute peat with compost of different origin (e.g. cattle manure, grape marc, green compost and sewage sludge) to grow foliage or flower ornamentals (Chen et al., 1988; Grigatti et al., 2007; Caballero et al., 2009; Larcher and Scariot, 2009) or with pumice, coconut fibers, coconut "peat" and pine bark (Larcher and Scariot, 2009). Municipal solid waste compost was also used as fertilizer for potted geranium production (Ribeiro et al., 2000).

Rice hulls are a rice milling industry byproduct and represent about 20% of the rice grain at harvest (Kamath and Proctor, 1998). Since rice is one of the major crops worldwide (FAO, 2009) it is available in large volumes (Kamath and Proctor, 1998; Del Amor and Gomez-Lopez, 2009) and at low cost. When whole rice hulls are used as substrates they are characterized by high air space when either fresh, carbonized or composted (Kampf and Jung, 1991; Garcia-C. et al., 2001; Evans and Gachukia, 2007). Ground rice hulls (GRH) instead present physical properties closer to peat (Sambo et al., 2008; Buck and Evans, 2010). Chemical characteristics of rice hulls have been studied by many authors (Kampf and Jung, 1991; Gachukia and Evans, 2008; Evans and Gachukia, 2008; Zanin et al., 2011). Rice hulls use permitted good results to be obtained in the growth of woody ornamental plants (Laiche and Nash, 1990; Garcia-C. et al., 2001; Marianthi, 2006), herbaceous ornamentals (Papafotiou et al., 2001; Evans and Gachukia, 2004) and tomatoes (Snyder, 1994) but more caution is required for peppers (Del Amor and Gómez-López, 2009) and pepper seedling production (Zanin et al., 2011). Soil-borne diseases, rice seed germination and release of toxic levels of Mn may be avoided using parboiled rice hulls (Einert, 1972).

Anaerobic digestion residues of the solid fraction of different organic materials

(Ward et al., 2008) are characterized by a high nutrient level, good organic matter content and low C/N ratio and may be used in agriculture as fertilizers or amendments (Salminen et al., 2001; Tambone et al., 2008) bringing numerous environmental and economic advantages (Braber, 1995). Compton and Zauche (2006a; 2006b) reported good results of anaerobic digestion biosolids, derived from cattle manure, as a substrate component but unfortunately, very little information is available on the use of anaerobic digestion residues (ADR) derived from plant byproducts as fertilizer in substrates.

The objective of this study was to evaluate the possibility of using GRH as alternative to peat, and ADR as fertilizer, for  $Rosa \times hybrida$  'Tilt Meillandina' rooting and cultivation.

#### Material and Methods

#### Substrates preparation

According to a 2-way factorial experimental design, 8 substrates were tested containing four different relative percentages of GRH (0, 33, 67 and 100% with the remainder being peat) and two levels (with and without) of an ADR deriving from fruit distillery wastes (20% of the final volume). ADR-free substrates were named 0-, 33-, 67- and 100- and those containing ADR 0+, 33+, 67+ and 100+, respectively. The GRH- and ADR-free (0-) substrate was considered as control. Physical and chemical characteristics of the substrates are described and discussed in chapter 4.

#### Agronomic evaluation of substrates

Thirty 10-cm (400 mL) plastic pots for each substrate were used for rooting and cultivation of *Rosa* ×*hybrida* 'Tilt Meillandina ' cuttings. Initial characterization of the cuttings is reported in table 1. Four single-node softwood cuttings per pot were used. Pots were transferred to a greenhouse and laid out on a bench with a completely randomized distribution. In order to maintain high humidity, the bench was covered with a plastic film for 30 days with an initial mist frequency of 10 sec, 6 times per day, then reduced to 10 sec twice per day. In the first phase, a shadecloth (50% shade) was placed over the bench. The shadecloth and plastic film were then removed. In this second phase, for a period of two weeks, mist was again increased to 10 sec 6 times per day; it was then interrupted and regular irrigation was applied as needed by each pot adopting the "look and feel" method (Niederholzer and Long, 1998; Raviv and Lieth, 2007).

On days 18, 23, 27 a foliar fertilization was applied using a 15N-2.2P-20.8K + microelements hydro-soluble fertilizer with an electrical conductivity of 1.2 mS cm<sup>-1</sup> (initial water EC = 0.400 mS cm<sup>-1</sup>). Beginning from day 30, three fertigations were applied per week using the same fertilizer as that used for foliar fertilization. Electrical conductivity of the nutrient solution was raised from 1.2 to 1.5 mS cm<sup>-1</sup> at day 80 of cultivation. Temperature ranged from 19.5 to 31.5 °C throughout the experiment.

During cultivation two pinchings were performed in order to stimulate secondary shoot formation; fresh and dry weight and dry matter percentage of pruned biomass were

determined.

The experiment was concluded when plants of the best performing treatment reached commercial standard, after 142 days of cultivation. At this point, percentage of rooted cuttings per pot and percentage of marketable pots were determined. Pots were considered marketable when at least three cutting per pot were rooted. On marketable pots, plant growth index [(height + widest width + perpendicular width) ÷ 3], SPAD value (SPAD 502, Konica-Minolta, Japan), flower bud number (including opened flowers), shoot number and leaf number were determined. Root, stem, leaf and flower bud fresh and dry weights, dry matter, and dry matter partitioning among plant organs were also evaluated.

#### Data statistical analysis

Data of commercial pot percentage were analyzed with the Chi-Square test. All other data were analyzed by analysis of variance (ANOVA) and means separated according to Student-Newman-Keuls test ( $P \le 0.05$ ). Values expressed as percentages were transformed prior to ANOVA analysis.

## Results

Results of analysis of variance showed significant "GRH  $\times$  ADR" interaction for many parameters. Among main effects, percentage of GRH was often significant, while fertilization with ADR rarely affected the different parameters (Table 2).

The fresh and dry weight of the biomass removed with pinching (Fig. 1 and 2) was the highest in the substrate containing only peat (6.07 and 1.46 g, respectively). Addition of 33% of GRH to the substrates resulted in lower weights of pruned material (-59.9 and - 48.5%, respectively for fresh and dry weight) but higher percentages of GRH did not lead to further decreases. Fertilization with ADR significantly reduced biomass of pruned materials of the 0% GRH substrate (-20.5 and -15.8%, respectively) but increased biomass of the other substrates even if this increase was not always significant (Fig. 1 and 2). Dry matter percentage of pruned biomass was only affected by main effects (Table 2 and Fig. 3). In the substrates containing 67% of GRH, the pruned biomass had lower dry matter on average than that obtained in GRH-free substrates. Furthermore, fertilization with ADR resulted in a higher dry matter (+9.05%; Fig. 3).

In ADR-free substrates, the percentage of rooted cuttings was reduced by increasing GRH content above 67% (-48.5%). Addition of ADR significantly reduced rooting percentage in substrates containing 0 and 33% of GRH (-22.7 and -27%, respectively), but did not affect rooting at higher percentages of GRH (Fig. 4). Percentage of marketable pots are, of course, affected by rooting percentage, but chi-square analysis indicated that only percentage of GRH in the substrate significantly affected this aspect (Table 2 and figure 5). In fact, the percentage decreased significantly only when relative percentage of GRH was 100% (80.0 vs. 32.3%).

Plant growth index of marketable pots with ADR-free substrates was gradually reduced with increasing GRH content and was 17.1% lower in 100- substrate compared to the control (Fig. 6). Fertilization with ADR improved the growth index and no differences were observed among substrates containing different percentages of GRH (Fig. 6).

Shoot and flower bud number was affected only by relative percentage of GRH in the substrate (Table 2). Increasing GRH content above 67%, shoot number was decreased by a 36.0% (Fig. 7). Instead, the negative effect of GRH on flower bud number was already significant at 67% GRH (-20.9%) (Fig. 8).

Leaf number gradually decreased with increasing percentages of GRH but only plants grown in the 100- substrate had fewer leaves than the control (-44.2%; Fig. 9). The highest SPAD value was observed in plants of the 33+ substrate (48.7). Compared to this, none of those grown in substrates containing GRH had a statistically different SPAD value, while in ADR-free substrates with percentages of GRH higher than 66% SPAD value was lower (-18.7%; Fig. 10).

Root fresh and dry weight was affected only by  $GRH \times ADR$  interaction (Table 2). In both cases, as for leaf number, compared to those of the control, plants grown in the 100substrate had lower values (-49.4 and -50.2%, respectively) (Fig. 11 and 12).

In ADR-free substrates, stem fresh weight decreased with 67% or more GRH in the substrate (Fig.13) and was less than half in 100- compared to the control (2.34 vs. 5.23 g). However, none of the substrates containing ADR resulted in a lower stem fresh weight than the control (Fig. 13). The results for dry weight of stems appeared to be slightly different. In fact, also in this case 67- and 100- substrates reduced stem weight compared to the control and no difference was observed among ADR-fertilized substrates but, instead, with 0+ and 33+ substrates, dry stem weights were lower than the analogous substrates without ADR fertilization (Fig. 14).

Leaf fresh weight was lowest in the 100- substrate but no significant difference was observed between leaf fresh weight obtained in the control substrate and that of any other substrate except for 100- substrate (Fig. 15). Instead, compared to the control (2.63 g), leaf dry weight was reduced in substrates containing 67% or more of GRH (-29.2 and -58.1% respectively for 67 and 100% GRH). None of the substrates fertilized with ADR had different values compared to the control (Fig. 16).

Fresh and dry flower bud weights were similarly affected by GRH  $\times$  ADR interaction (Table 2); in fact, while in ADR-free substrates flower bud weights were similar (on average 2.16 and 0.374 g, respectively) and lower than those obtained in the control (3.75 and 0.597 g), in ADR-fertilized substrates flower bud weights were both similar and not different from those of the control (on average 3.38 and 0.538 g) (Fig. 17 and 18).

Response of the whole fresh and dry weights to treatments obviously reflected the response of the different plant organs. For both parameters, increasing percentages of GRH to 67 in ADR-free substrates resulted in a significant reduction of plant weight (-25.0 and -

26.5%), which further decreased at the highest percentage of GRH (-54.7 and -55.8%). Fertilization with ADR in the 0% GRH substrate slightly reduced plant weights but GRH-containing substrates had similar results compared to the 0+ substrate and were not different from those obtained with the control (Fig. 19 and 20).

Percentages of dry matter in roots, stems, leaves, flower, as well as in the whole plant were not affected by treatments (Table 2) and were, on average, 9.35, 24.3, 21.5, 16.2 and 17.6% respectively.

Lastly, dry matter partitioning among plant organs (Fig. 21) was affected by both main factors. All substrates containing GRH had relatively higher root dry matter and lower stem dry matter compared to those of the control. While relative dry mater of root, leaves and flowers were not affected by ADR fertilization, plants grown in ADR-fertilized substrates had a lower relative stem dry matter (Fig. 21).

#### Discussion

The percentage of rooted cuttings showed some differences compared to observations in the rooting experiment of 'La Sevillana' rose (chapter 4). Higher rooting percentages in substrates 0- and 33- were obtained in both experiments, but in this case substrate 67- allowed a higher rooting percentage compared to that observed for 'La Sevillana' rose. Furthermore, in both cases substrates with 100% GRH and without ADR induced lower rooting percentages compared to that of the control but, here, the differences were much more important. ADR fertilization had a negative effect on rooting, even if this did not reduce the percentage of marketable pots. This is in contrast with what was observed in 'La Sevillana' rose on which, in the early stage of rooting, it had a positive effect. However, the results are consistent with the detrimental effect of high salinity in rooting substrates observed by several authors (Bertram, 1991; Iglesias-Díaz et al., 2009), and are much closer to those of geranium (chapter 4), even if type of cutting and species are very different (shoot-tip herbaceous cuttings for geranium and single-node softwood cuttings for rose).

In 100% GRH substrates, the one with the highest air filled porosity, rose showed great difficulty in producing adventitious roots. In a rooting experiment on baldcypress, Copes and Randall (1993) reported that excessive aeration in media may be negative for the rooting process. King et al. (2011) in a baldcypress rooting experiment explained that too

high aeration may delay rooting process after callus formation and that substrates with higher water holding capacity (e.g. 0 and 33% GRH) may have favored root growth. Rein et al.(1991) also underlined the importance of moisture in propagation medium to produce adventitious roots.

Among all considered parameters, those regarding aboveground organs (number, weights or partitioning of different organs, SPAD value), in the absence of ADR, resulted as negatively affected by percentages of GRH higher than 33. This is consistent with a series of other experiments. Evans and Gachukia (2004) reported decreasing value for dry shoot weight of geranium, tomato, impatiens and marigold cultivated in substrates where peat was progressively substituted by fresh parboiled rice hulls up to 40%, even though not significant differences were observed. Pepper seedlings cultivated in substrates containing fresh rice hulls had reduced growth (Lee et al., 2000a, 2000b). Zanin et al. (2011) observed growth reduction of tomato and pepper seedlings cultivated in substrates with increasing GRH rate, while chicory seedlings growth was not affected, or even enhanced, by the presence of GRH. *Salvia splendens* and *Begonia semperflorens* seedlings resulted as negatively affected by increasing rate of GRH (chapter 2) and again potted geranium and 'La Sevillana' rose were negatively affected by percentages of rice hulls higher than 33%, when ground (chapter 5).

As highlighted in previous chapters, and according to Zanin et al. (2011), these decreasing values are probably linked to poorer physical properties of substrates with high rates of GRH, which in some cases had too much air space and low water holding capacity and, in particular, low easily available water. Although plants were watered daily to suit the physical properties of each substrate, some water stress may have occurred, consequently causing this reduction in values. Oh et al. (2007) linked the reduction of some biometric parameters in kalanchoe, when water supply was reduced, to physical properties of different substrates. Increasing root parameters (weights, dry mass partitioning), consequent to increasing of GRH rate in substrates, were also observed in potted geranium (chapter 5). This may partly support the theory of water stress due to lower physical properties. In fact, within certain limits, plants in water stress stimulate root production to better use available water (Taiz and Zeiger, 1998).

Considering plant growth, ADR addition, as main factor, generally did not have a

significant effect on the considered parameters. When significant, the effect was positive as ADR improved fresh and dry weight of initial plant growth (material removed with pinching), SPAD value, flower bud fresh and dry weight. However, ADR effect was remarkable, as highlighted by the numerous GRH × ADR interaction effects. ADR fertilization of 0% GRH often decreased plant performances compared to the ADR-free control (0-), which, however, rarely resulted as being significant. Other than that, ADR addition was positive on substrates with high GRH contents (67 and 100%) and improved plant growth, compared to that obtained in the homologous substrates without ADR, and performance were often similar to that in 0+ and control substrate. The beneficial effect of ADR on 'La Sevillana' rose was, partially, also observed in the cutting rooting (chapter 4) and cultivation experiments (chapter 5) where, when significant, the effect of ADR was positive. A more evident positive effect of ADR addition was observed in the ornamental seedling experiment (chapter 2) and on tomato seedlings (chapter 3). The positive effect of ADR addition is, at least partially, due to its slightly positive effect on improving physical characteristics of substrates with high GRH percentages, as demonstrated in the previous experiments (chapters 2, 3, 4 and 5). However the positive effect of ADR is mainly due to the higher nutrient level content (see also chapters 2, 3, 4 and 5). In fact, SPAD value, which is related to higher chlorophyll content and, hence, to a higher nitrogen availability (Wood et al., 1992; Duce et al., 1997), confirmed the positive effect of ADR on plant nutrition, which is probably linked to both an increase of nutrient level and also mitigation of nutrient unbalance in substrates with high rates of GRH (see also chapters 2, 3, 4 and 5). A positive effect in ornamentals cultivation due to higher nutritional level of decomposed biomasses was observed by several authors (Atiyeh et al., 2001; Garcia-Gomez, 2002; Ostos et al., 2008; Herrera et al., 2008). However, the positive effect of ADR probably occurred during growth after rooting, when salinity tolerance and nutritional needs are higher, as observed by Bertram (1991) who worked with Hibiscus rosa-sinensis.

## Conclusions

Rooting and cultivation results in 'Tilt Meillandina' rose highlighted the negative effect of increasing GRH content on rooting percentage, and thus, on number of marketable pots, and on growth parameters. However it is possible to partially substitute peat with 33% GRH by volume because when differences were observed they were minimal and not easily noticeable in practice. Many parameters showed a positive effect of ADR fertilization when associated with high rates of GRH, with performances similar to those of the control substrate. Unfortunately ADR appeared to be a limiting factor during cutting rooting phase. Hence, in order to reduce peat utilization in substrates for rooting and cultivation 'Tilt Meillandina' rose, GRH can be utilized at a relative rate of 67% and 33% of peat, combined with 20% of ADR, which allows a reduction of about 70% of the amount of peat used.

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# Tables

Table 1	. Initial	characterization	n of the	rose cuttings	used in th	e experiment.
I able I	• IIIItitai	. chui actei izatioi	i or the	105c cuttings	ubcu m u	ie experiment.

	Rosa
Stem diameter (mm)	2.68
Stem length (mm)	22.66
Leaf number	1.0
Leaf fresh weight (mg)	225
Stem fresh weight (mg)	147
Total cutting fresh weight (mg)	372
Leaf dry weight (mg)	63
Stem dry weight (mg)	37
Total cutting dry weight (mg)	100
Leaf dry matter (%)	27.57
Stem dry matter (%)	25.51
Total cutting dry matter (%)	26.81

	GRH	ADR	$\operatorname{GRH} \times \operatorname{ADR}$
Rooted cuttings (%)	***	**	***
Marketable pots (%)	*	n.s.	n.s.
Pruned biomass fresh weight	***	**	***
Pruned biomass dry weight	***	**	***
Pruned biomass dry matter	**	*	n.s.
Plant growth index	**	n.s.	***
Shoot number	**	n.s.	n.s.
Flower bud number	*	n.s.	n.s.
Leaf number	**	n.s.	*
SPAD value	***	***	*
Root fresh weight	n.s.	**	***
Stem fresh weight	***	n.s.	***
Leaf fresh weight	***	n.s.	***
Flower bud fresh weight	n.s.	*	*
Total fresh weight	**	*	***
Root dry weight	n.s.	n.s.	***
Stem dry weight	**	**	***
Leaf dry weight	***	***	***
Flower dry weight	n.s.	n.s.	*
Total dry weight	***	***	***
Root dry matter	n.s.	n.s.	n.s.
Stem dry matter	n.s.	n.s.	n.s.
Leaf dry matter	n.s.	n.s.	n.s.
Flower bud dry matter	n.s.	n.s.	n.s.
Aerial part dry matter	n.s.	n.s.	n.s.
Total dry matter	n.s.	n.s.	n.s.
Root (%)	***	***	n.s.
Stem (%)	n.s.	n.s.	n.s.
Leaf (%)	*	*	n.s.
Flower (%)	n.s.	n.s.	n.s.

Table 2. Main effect GRH and ADR and interaction "GRH × ADR" at the end of the experiment.

Values with the same letter are not different according to Tukey's HDR test (P $\leq$ 0.05). \*\*\*, \*\* and \* = significant at P  $\leq$  0.001, 0.01 and 0.05, respectively. n.s. = not significant.

# **Figures**



Figure 1. Effect of interaction "GRH × ADR" on fresh weight of pruned biomass at pinching. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 2. Effect of interaction "GRH × ADR" on dry weight of pruned biomass at pinching. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 3. Main effects of relative rate of ground rice hulls (GRH) content and ADR fertilization (+) or not (-) on dry matter of pruned biomass at pinching. Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 4. Effect of interaction "GRH × ADR" on the percentage of rooted cuttings. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 5. Effect of relative rate of ground rice hulls (GRH) on percentage of marketable pots. Different letters indicate significant differences according to Chi-Square (P≤0.05). Vertical bars represent ± SE of means.



Figure 6. Effect of interaction "GRH × ADR" on plant growth index. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 7. Effect of relative rate of ground rice hulls (GRH) on shoot number. Different letters indicate significant differences according to Chi-Square (P≤0.05). Vertical bars represent ± SE of means.



Figure 8. Effect of relative rate of ground rice hulls (GRH) on flower bud number. Different letters indicate significant differences according to Chi-Square (P≤0.05). Vertical bars represent ± SE of means.



Figure 9. Effect of interaction "GRH × ADR" on leaf number. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 10. Effect of interaction "GRH × ADR" on SPAD value. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 11. Effect of interaction "GRH × ADR" on root fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 12. Effect of interaction "GRH × ADR" on root dry weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 13. Effect of interaction "GRH × ADR" on stem fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 14. Effect of interaction "GRH × ADR" on stem dry weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 15. Effect of interaction "GRH × ADR" on leaf fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 16. Effect of interaction "GRH × ADR" on leaf dry weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 17. Effect of interaction "GRH × ADR" on flower bud fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 18. Effect of interaction "GRH × ADR" on flower bud dry weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.


Figure 19. Effect of interaction "GRH × ADR" on total fresh weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 20. Effect of interaction "GRH × ADR" on total dry weight. (GRH = relative rate of ground rice hulls; ADR = anaerobic digestion residues fertilization [+] or not [-]). Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.



Figure 21. Main effects of relative rate of ground rice hulls (GRH) content and ADR fertilization (+) or not (-) on root, stem, leaf and flower repartition of biomass. Different letters indicate significant differences according to SNK test (P≤0.05). Vertical bars represent ± SE of means.

Chapter VII

Conclusions

## Conclusions

In this study, different substrates were prepared in order to evaluate suitability of rice hulls and anaerobic digestion residues for vegetable and ornamental transplant (chapters 2 and 3), ornamental rooted cuttings (chapter 4) potted ornamentals (chapter 5) and rooting and cultivation of small size potted rose (chapter 6) production.

Physical characteristics of different substrates were modified, sometimes heavily, by rice hulls content in substrates. Rice hulls have increased total pore space in substrates for seedling production and air pore space in all substrates, sometimes over acceptable limits. This have caused the reduction of water holding capacity in all substrates of the different experiments and in particular of easily available water and of water buffer capacity of mixes prepared for seedling and rooted cutting production. Rice hulls physical characteristics were worsen when whole rice hulls were used compared to the 2 mm ground rice hulls in substrates for potted ornamental production. These observation suggest a change of water management adopting frequent but lower water volumes applications. Anaerobic digestion residues had showed a slightly positive effect on physical characteristics but generally not sufficient to overcome negative effect of rice hulls.

Also chemical characteristics of substrates were significantly affected by increasing rice hulls content. Increasing rate of rice hulls in substrates raised up P and K availability and reduced cation exchange capacity and nitrogen (total nitrogen and nitrate nitrogen content) Ca and Mg availability, pointing out a potential nutritional imbalance. Fertilization with anaerobic digestion residues restored the nutritional balance caused by increasing rice hulls rate and generally increased nutrient content. Even though significant effect were observed on substrates pH and electrical conductivity values ranged generally between optimal values.

Under agronomical aspect rice hulls use as peat alternative resulted possible for tomato and *Salvia officinalis* seedling and geranium rooted cutting production. These seedling tolerated without negative effect up to 67% peat substitution with rice hulls if anaerobic digestion residues were added in substrates but good results obtained for tomato in the first study were re-dimensioned to a maximum rate of 33% rice hulls in substrates in the second one. Geranium rooted cutting production resulted possible with a total peat substitution but strictly without anaerobic digestion residues to avoid reducing rooting percentage. Partial peat substitution with 33% of rice hulls resulted possible in potted geranium, rose and in rooting and direct cultivation of small roses if substrate were fertilized with anaerobic digestion residues Unfortunately peat substitution with rice hulls is not recommended for longer ornamentals cultivation cycle of seedling (*Salvia splendens* and *Begonia semperflorens*) and rose cutting. In those cases fertilization with anaerobic digestion results on seedlings production but not sufficient while in rose cutting experiments decreased rooting percentages.

This study pointed out the technical possibility to partially and sometimes totally substitution of peat with rice hulls and anaerobic digestion residues meeting potential economic and environmental benefits. On the other hand, some potential issues regarding crop management raised up. In this study, in fact, was demonstrated that rice hulls use requires different water management and sometimes higher water amount, it requires the rice weed management too and should consider potential higher percentage of not marketable product.

Only considering all these positive and potential negative elements rice hulls and anaerobic digestion residues use can assure good results in a sustainable approach.

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