AGRIVITA VOLUME 36 No. 2 JUNE - 2014 ISSN : 0126-0537

ACCELERATION ON THE GROWTH OF RUBBER PLANTING MATERIALS BY USING FOLIAR APPLICATION OF HUMIC ACID

Andi Nur Cahyo*), Risal Ardika, Jamin Saputra and Thomas Wijaya
Sembawa Research Centre, Indonesian Rubber Research Institute
Po Box 1127, Palembang 30001, South Sumatra, Indonesia
*)Corresponding author Phone:+62-85292011670 Email : nurcahyo.andi@yahoo.co.uk

Received: May 6, 2014 /Accepted: July 12, 2014

ABSTRACT

The best rubber planting materials are needed to build the best rubber plantation. Humic acids could be used to improve the growth of rubber planting materials. Humic acid plays a role as a hormone-like substance. This research was aimed to determine the optimal concentration of foliar application of humic acid in order to enhance the growth of rubber tree planting materials. This research was arranged in a completely randomized block design with five treatments and four replicates. The treatments were the concentrations of humic acids, i.e. 0; 250; 500; 750; and 1,000 ppm. Observations were made on rubber tree diameter, plant height, shoot and root biomass, and nutrient content of leaves and the stem. The results showed that foliar application of 1,000 ppm of humic acids could enhance the growth of rubber tree planting materials. Foliar application of 500 – 1,000 ppm of humic acids could increase K content of the stem. The effects of foliar application of humic acids were more apparent in the root part than in the shoot part.

Keywords: *Hevea brasiliensis*, humic acid, growth, hormone, nutrient uptake

INTRODUCTION

Natural rubber is a commodity that plays important role for Indonesia's income, since it was a source of foreign exchange reaching US$ 2.96 billion in 2010 (FAO, 2012), providing income and livelihood to above 11 million people particularly in Sumatra and Kalimantan, and also needed to preserve the environment (Sembawa Research Centre, 2008). Indonesia's natural rubber production is about 3.08 million tons per year (FAO, 2013), placing Indonesia in the second biggest natural rubber producer in the world after Thailand despite the fact that the area of Indonesia's rubber plantation is the biggest. It means that rubber plantation productivity in Indonesia is still low. This condition happened because in Indonesia there are a lot of old rubber plantations and the adoption of high yielding clone is still low. Therefore, to increase Indonesia's rubber production, replanting and good planting materials are required almost in all areas of rubber plantation in Indonesia

Based on the average of expansion of rubber plantation area in Indonesia that reaches more than 10,000 ha/year, more than six millions rubber planting materials are needed for replanting in Indonesia (Directorate General of Estates, 2011). To fulfil this demand, a lot of work is needed to produce high quality of rubber planting materials. This quality is needed in order to fulfil Indonesian National Standard of rubber planting materials. To produce a high quality of rubber planting materials, some efforts have to be done, one of which is through foliar application of humic acid in order to improve the growth of rubber planting materials. Some research work mentioned that humic acid could play a role as an auxin-like hormone.

Humic acids are organic materials obtained as final process of decomposition of plant and animal for million years in the soil (Albayrak and Camas, 2005). These substances are available in the stabilized soil organic matter and humus as natural organic poly electrolytes. These substances are important as their role in the regulation of a lot of biological and chemical processes occurring in natural ecosystem (Chen *et al.*, 2004; Nardi *et al.*, 2002), including their affect on the growth of plants (Arancon *et al.*, 2003). Some research proposed that the improvement of plant growth resulted from the

Accredited SK No.: 81/DIKTI/Kep/2011

http://dx.doi.org/10.17503/Agrivita-2014-36-2-p112-119

function of humic substance as soil conditioner that improves the soil bioavailability of some nutrients (iron and zinc) (Chen *et al*., 2004), but other research also found that humic substances could also affect plant metabolism directly (Nardi *et al*., 2002).Foliar application of humic acids also improvesthe growth of some plant species, such as tomatoes, cottons, and grapes (Brownell *et al*., 1987; Fernández-Escobar *et al.*, 1996). Fernández- Escobar *et al.* (1996) also found that K status increased significantly. Besides, leaf Ca, Mg, B, and Fe concentrations also significantly increased.

The positive effect of humic substances on plant metabolism isexerted on cell membrane function and stimulates nutrient uptake (Visser, 1986; Varanini and Pinton, 1995; Nardi *et al.,* 2002). It also affects the growth of the plants as hormone-like substances (Bottomley, 1917; Hillitzer, 1932; Rerabek, 1963; Cacco and Dell'Agnola, 1984; Muscolo*et al*., 1998).Some research showed that humic acid might increase plant growth (Vaughan and Malcolm, 1985; Varanini and Pinton, 2001; Vaccaro et al., 2009; Mora et al., 2010; Trevisan *et al*., 2011). Based on research done by Arancon*et al.* (2003), humic acid from cow manure could increase the root dry weight of marigolds and strawberry significantly at the concentration of 500 mg/kg. Besides, humic acid from vermicompost at concentration of 250 – 1,000 mg/kg could also increase the root dry weight of tomatoes. Atiyeh *et al*. (2002) found that usually at low concentration (50 – 500 mg/kg), humic acid could increase the plant growth, but when it was applied at higher rate (500 – 1,000 mg/kg) it could not increase the plant growth significantly.The growth curves on the effect of application of humic acids show climbing growth with increasing rates of humic acids until reaching the peak and then it usually decreases at higher rates of the humic acids (Chen and Aviad, 1990; Atiyeh *et al*., 2002).

Foliar application of humic acid stimulates the growth of young olives shoot when they are grown without additional mineral elements (Fernández-Escobar *et al.*, 1996).The plant growth stimulating activity revealed by some research seems to be generated by hormone-like materials available in humic acids (O'Donnell, 1973; Casenave de Sanfilippo *et al*., 1990; Fernández-Escobar *et al.*, 1996).

Merlo *et al*. (1991) and Trevisan *et al*. (2011) proved that humic substances could increase photosynthetic metabolism in maize leaves. It has been hypothesized that humic substances probably affected plant physiology as an auxin-like hormone. Some research showed that humic substances could enhance the growth of plants as an auxin-like hormone. Research on earthworm humic substances on the growth of some crops showed that humic acid enhance plant growth by increasing the uptake of anion and cation, synthesis of proteins and nitrate metabolism enzymes (Dell'Agnola and Nardi,1987; Muscolo *et al*., 1999). Muscolo *et al*. (1999) also said that the stimulation effect of humic acid on the growth of plants is probably because of hormone-like activity, especially auxin-like activity of humic substances. Furthermore, humic substances have also been found to promote plant growth in gibberellins, cytokinin, and auxin bioassays (Phuong and Tichy, 1976; Atiyeh *et al.*, 2002).

MATERIALS AND METHODS

This research was conducted from May 2011 to February 2012 in the glass house of Sembawa Research Centre. The experimental layout was a Randomized Completely Block Design with five treatments. Each treatment was replicated into four replicates. The treatments were foliar application of 0(control); 250; 500; 750; and 1,000 ppm of humic acids. The treatments were applied to the leaves of each whorl after they reached maturation (indicated by its dark green colour).

The type of rubber planting materials used in this experiment was rubber budded stump grown in 25 x 40 cm polybags. The planting media was sandy subsoil poor of nutrition. The observation was conducted on plant biomass, nutrition uptake (N, P, K, Ca, and Mg), and some other growth parameters (stem diameter, plant height, and number of leaves). Observation on plant biomass and nutrition uptake (N, P, K, Ca, and Mg) was done when the planting material reached the age of 6 months (destructive observation). Growth parameters such as stem diameter at 5 cm above grafting union and plant height were observed once in two weeks. Maintenance of planting materials was done by daily irrigation and application of fungicide to prevent disease attack when the symptoms were detected. Chemical fertilizer was also given once in a month at recommended dose.

The results of the observation were analyzed using ANOVA of Completely Randomized Block Design and Duncan Multiple Range Test to differentiate between treatments using SAS 9 software (SAS Institute Inc., 2002).

RESULTS AND DISCUSSION

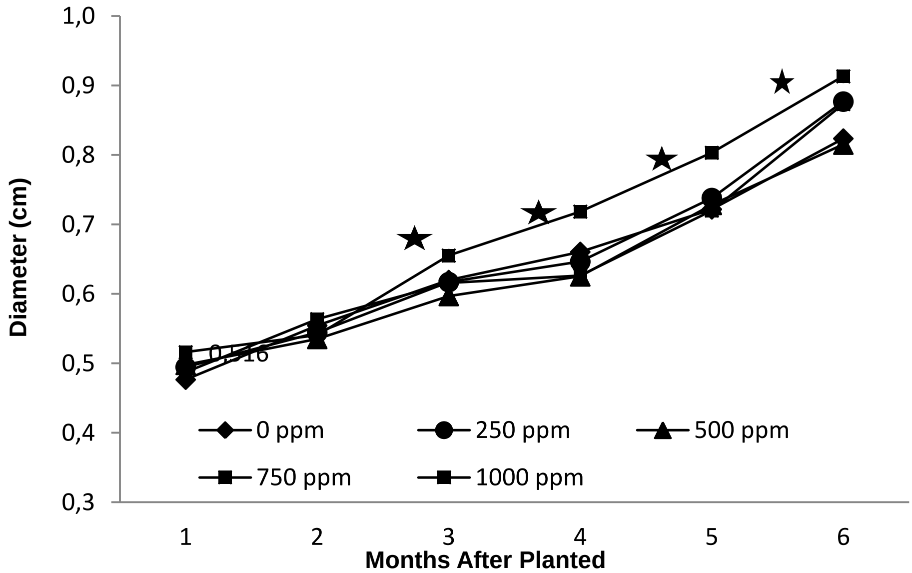
Plant Growth

Generally, the effect of foliar application of humic acid on rubber tree planting materials significantly appeared on the rubber tree diameter. The effect of humic acid on rubber tree diameter was significant at concentration of 1,000 ppm. This phenomenon is presented in Figure 1 showing that from 0 – 2 months after planting, the diameter of rubber tree was not significantly different. It could happen because the first foliar application of humic acid had been given when the age of rubber trees were 2 months old, when the leaves were mature. After the foliar application of humic acid was given, the diameter of rubber trees treated with 1,000 ppm of humic acid grew faster than those of other treatments; therefore, the diameter was the highest (Table 1). It shows that foliar application of humic could affect the growth of rubber tree quickly.

Table 1.Effect of foliar application of humic acid on rubber tree diameter at 6 months after planted

Treatments / Concentration of humic acids (ppm)	Rubber tree diameter (cm)	Rubber tree height (cm)
0	0.82 b	62.86 a
250	0.87 ab	61.62 a
500	0.81 b	57.13 ab
750	0.88 ab	50.11 b
1,000	0.91 a	66.17 a

Remarks: Values followed by the different letters in the same column are significantly different at 95% confidence interval of Completely Randomized Block Design



Remarks: Lines separated by stars (★) are significantly different with control (0 ppm) at 95% confidence interval of Completely Randomized Block Design

Figure 1. The effect of humic acid on rubber tree diameter

This result is in accordance with the research done by Muscolo *et al*. (1993); Nardi*et al*. (2002) confirmed that humic substances improves plant growth and development. Tan and Tantiwiramanond (1983); Atiyeh *et al*. (2002) reported that shoots, roots, and nodules dry weight of soybean, peanut, and clover plants have been improved after application of humic acids.

The improvement of the diameter of rubber tree might be induced by the presence of humic acids as plant growth hormone-like substances (Vaughan and Malcolm, 1985; Nardi *et al.,* 1996; Nardi *et al*., 2002).This opinion is in accordance with the research result done by Muscolo *et al*. (1999) who found that humic acids treatment on carrot cells improved their growth and promoted morphological growth and development.The role of humic acids is mainly exerted on cell membrane functions and promotes nutrient uptake (Visser, 1986; Varanini and Pinton, 1995; Nardi *et al*., 2002). Furthermore, Bottomley (1917); Hillitzer (1932); Rerabek (1963); Cacco and Dell'Agnola (1984), and Muscolo *et al*. (1998) confirmed that humic substance showed auxin-like activity, but they found that humic acids' effect is not as strong as plant growth hormone and they found that humic acids are not the same as auxin. The role of humic acids, are not only as auxin-like, but also gibberellins-like hormone (Nardi *et al*., 1991; Muscolo *et al*., 1999; Nardi *et al*., 2002). Furthermore, humic acids have been known to be able to induce plant growth and the development in auxin, gibberellin and cytokinin test (Phuong and Tichy, 1976; Atiyeh *et al*., 2002). In Table 1 it is highlighted that although humic acids could enhance the diameter of rubber tree, the height did not increase. It indicates that the height of rubber tree planting materials was more affected by endogen auxin than exogen auxin-like from humic acids. Furthermore the highest diameter of 1,000 ppm treatment might be caused by the role of humic acids as gibberellin-like hormone instead of auxin-like hormone. Therefore, this treatment could enhance the diameter of rubber trees but not the height of rubber trees.

Figure 1 also shows that other treatments other than that of 1,000 ppm foliar application of humic acid, did not show any significant difference from the control (foliar application of 0 ppm humic acid). This phenomenon could happen because the effectiveness of foliar application of humic acids is in a certain range of concentration of humic acids. Atiyeh *et al*. (2002) found that the plant growth was improved after the plants were treated with humic acid at concentration of 50–500 mg/kg. However, it significantly reduced the plant growth when the concentration wasmore than 500 mg/kg. Different result reported by Valdrighi *et al*. (1996) emphasized that humic acids could enhance the growth of chicory plants when the concentration was equal to or greater than 1,000 mg/kg. The different optimum concentration of humic acid might be influenced by the kind of the plants. From Figure 1, it can also be concluded that the optimum concentration of humic acids for foliar application on rubber trees was at least 1,000 ppm. The effectiveness of humic acids application above 1,000 ppm needs to be studied further.

The similar results were found on the root and rubber tree biomass parameters. On these parameters, 1,000 ppm foliar application of humic acids could improve rubber tree and root biomass. This treatment could only affect rubber tree and root biomass, but did not affect other parameters like shoot biomass and root-shoot ratio (Table 2).These phenomena indicate that application of humic acids affected root part more than the shoot part. These results were in line with research result by Vaughan and Malcom (1985); Nardi *et al*. (2002); and Atiyeh *et al*. (2002). They found that the effect of humic acids was higher on the root part than the shoot part.

The quicker growth of root part than shoot part as the application of 1,000 ppm of humic acids might happen because humic acid contains auxin-like and cytokinin-likehormones (Nardi *et al*., 1998) which can improve root growth. The auxin hormone could promote root initiation whether the cytokinin could enhance the root growth Gardner *et al*. (1985). Although the humic acids were spread on the leaves, they were absorbed by the leaves and distributed to the root part. So, they promoted higher rate of root growth.

Table 2. Effect of foliar application of humic acid on some rubber tree growth parameter at 6 monthsafter planting

Treatments / Concentration of humic acids (ppm)	Growth Parameters			
	Rubber tree biomass (g)	Root biomass (g)	Shoot biomass (g)	Root-shoot ratio
0	34.21 bc	09.26 bc	24.95 abc	0.37 a
250	38.29 ab	11.69 a	26.59 ab	0.46 a
500	27.01 c	07.63 c	19.38 c	0.40 a
750	32.11 bc	10.21 ab	21.90 bc	0.47 a
1,000	42.73 a	12.58 a	30.14 a	0.42 a

Remarks: Values followed by different letters in the same column are significantly different at 95% confidence interval of Completely Randomized Block Design

Table 3. Effect of foliar application of humic acid on leaves nutrient status at 6 months after planting

Treatments / Concentration of humic acids (ppm)	Leaves nutrient content (%)				
	N	P	K	Ca	Mg
0	3.84 a	0.23 a	1.12 a	0.95 a	0.19 a
250	3.72 a	0.21 a	1.18 a	0.86 a	0.18 a
500	3.67 a	0.24 a	1.10 a	1.05 a	0.22 a
750	3.58 a	0.21 a	1.07 a	0.92 a	0.19 a
1,000	3.72 a	0.22 a	1.20 a	1.35 a	0.20 a

Remarks: Values followed by the different letters in the same column are significantly different at 95% confidence interval of Completely Randomized Block Design

The high rate of root growth also influenced the growth of the stem. Gardner *et al*. (1985) said that the root system of a tree produces gibberellins hormone. This hormone enhances the growth of the plant internodes; it can improve the growth of dwarf plants (Gardner *et al*., 1985). Therefore the high biomass of the root system of treated rubber trees with 1,000 ppm of humic acids affected the improvement of the diameter of this treatment.

For the shoot part, humic acids did not show significant effect on the shoot biomass parameters. Humic acids only improved the diameter, not the height of rubber tree (Table 1); therefore, the shoot biomass of treated trees had no significant difference from the control. This phenomenon might happen becausethe height of the plants was more influenced by endogenous auxin than by exogenous auxin-like from humic acids. Nevertheless, the humic acids affected the root and shoot lateral growth more than the shoot apical growth.

Table 2 also shows that root-shoot ratio of all treatments were not significantly different. It could happen because when the growth of the roots was improved, the growth of the shoots was also improved due to the improvement of nutrient uptake ability as the increment of root system. This condition also impacted the rubber tree biomass. The higher biomass of root system resulted in the higher biomass of rubber tree (Table 2). So, the ratio of root and shoot of all treatments between treatments were similar.

Nutrient Status

In addition to growth parameter, observation was also conducted on the nutrient status of the leaves. The results of the nutrient status observation are presented in Table 3.Table 3 shows that foliar applications of all concentration of humic acids did not affect the nutrient status (N, P, K, Ca, and Mg) of the leaves. This fact indicates that humic acids were not enriching nutrient content of the leaves, but they acted as hormone-like substances.

The different results were found on the observation of nutrient status of the stem. Nutrient status of the stems especially for N and K was affected by humic acids treatments. These results are presented in Table 4 showing that foliar application of humic acid at concentration of 500 ppm could increase N content of the stems. Besides, foliar application of humic acid at concentration of 500 – 1,000 ppm could increase K content of stems.

Table 4. Effect of foliar application of humic acid on stems nutrient status at 6 months after planting

Treatments / Concentration of humic acids (ppm)	Stems nutrient content (%)				
	N	P	K	Ca	Mg
0	1.27 b	0.17 a	1.41 d	1.73 a	0.14 a
250	1.08 b	0.15 a	1.51 cd	1.79 a	0.12 a
500	1.80 a	0.20 a	2.04 b	1.44 a	0.18 a
750	1.09 b	0.16 a	1.86 bc	1.62 a	0.13 a
1,000	1.33 b	0.17 a	2.47 a	1.94 a	0.15 a

Remarks: Values followed by the different letters in the same column are significantly different at 95% confidence interval of Completely Randomized Block Design

These results were different from those of nutrient content of the leaves. These facts indicate that humic acids did not affect the enhancement of nutrient status directly, but they could enhance the nutrient status by affecting cellular metabolism of the plants as the role of auxin. Valdrighi *et al*. (1996) reported that humic acids could enhance uptake of mineral nutrients by increasing the permeability of the root cell membranes. In addition, Gardner *et al*. (1985) explained that auxin could enhance proton exchange, membrane capacity, and potassium uptake. Therefore, humic acid could also increase K uptake, and then K content of stems was enhanced (Table 4).

Table 4 also shows that foliar application of 500 ppm of humic acid could increase N content of rubber tree stems. This result was in accordance with the result of a research conducted by Tattini*et al*. (1991). It was reported that N uptake rate by olive plants roots wasincreased after treated by humic acids at concentration ranging from 30–120 mg/pot. Concentration of humic acids above this range reduces N uptake of the plants. It indicates that humic acids positively affect N uptake in a certain range of concentration.

CONCLUSIONS

The application of 1,000 ppm of humic acids could enhance the growth of rubber tree planting materials. Furthermore, foliar application of 500 – 1,000 ppm of humic acids could increase K content of the stem. In addition, the effects of foliar application of humic acids were more apparent in the root part than in the shoot part. Finally, it is recommended that further research needs to be carried out to study the effect of foliar application of humic acids at higher concentration.

REFERENCES

Albayrak, S. and N. Camas. 2005. Effect of different levels and application times of humic acid on root and leaf yield components of forage turnip (*Brassica rapa* L.). *Journal of Agronomy* 4(2): 130-133.

Arancon, N.Q., S. Lee, C.A. Edwards, and R.Atiyeh. 2003. Effects of humic acids derived from cattle, food and paper-waste vermicomposts on growth of greenhouse plants. *Pedobiologia* 47: 741-744.

Atiyeh, R.M., S. Lee, C.A. Edwards, N.Q. Arancon and J.D. Metzger. 2002. The influence of humic acids derived from earthworm-processed organic wastes on plant growth. *Bioresource Technology* 84: 7-14.

Bottomley, W.B. 1917. Some effects of organic-promotion substances (auxinomones) on the growth of *Lemna minor* in mineral cultural solutions. *Proceedings of the Royal Society of London Series B – Biological Sciences* 89: 481-505.

Brownell, J.R., G. Nordstrom, J. Marihart, and G. Jorgensen. 1987. Crop responses from two new leonardite extracts. *Sci.Total Environ*. 62: 491-499.

Cacco G. and G. Dell'Agnola. 1984. Plant growth regulator activity of soluble humic complexes. *Canadian Journal of Soil Science* 64: 225-228.

Casenave de Sanfilippo, E., J.A. Argiiello, G. Abdala and G.A Orioli. 1990. Content of auxin-, inhibitor- and gibberellin-like substances in humic acids. *Biol. Plant.* 32: 346-351.

Chen, Y. and T. Aviad. 1990. Effects of humic substances on plant growth. In: Mac

Carthy, P., Clapp, C.E., Malcolm, R.L., Bloom, P.R. (Eds.), Humic Substances in Soil and Crop Sciences: Selected Readings. ASA and SSSA, Madison, WI. p. 161-186.

Chen Y., M. De Nobili and T. Aviad. 2004. Stimulatory effects of humic substances on plant growth. Soil Organic Matter in Sustainable Agriculture. Boca Raton, Florida: CRC Press. p. 103–129.

Dell'Agnola, G. and S. Nardi. 1987. Hormone-like effect and enhanced nitrate uptake induced by depolycondensed humic fractions obtained from *Allolobophora rosea* and *A. caliginosa* faeces. *Biology and Fertility of Soils* 4: 115-118.

Directorate General of Estates. 2011. *Tree Crop Estate Statistics of Indonesia*. Jakarta.

FAO. 2012. *FAO STAT*. http://faostat.fao.org/site/339/default.aspx. Accessed on October 16, 2012.

FAO. 2013. *FAO STAT*. http://faostat.fao.org/site/339/default.aspx. Accessed on April 24, 2012.

Fernández-Escobar, R.M. Benlloch, D. Barranco, A. Duefias and J.A. GutérrezGañán. 1996. Response of olive trees to folk application of humic substances extracted from leonardite. *Scientia Horticulturae* 66: 191-200

Gardner,F.P.,R.B Pearce and R.L. Mitchell. 1985. Physiology of crop plants. Iowa State University Press. p.327.

Hillitzer A. 1932. Über den einfluss der humus toffee auf das wurzelwachstum *Beihefte zum Botanischen Zentralbatt.* 49 : 467 – 480

Merlo, L., R. Ghisi, N. Rascio and C.Passera. 1991. Effects of humic substances on carbohydrate metabolism of maize leaves. *Can. J. Plant Sci.* 71: 419-425

Mora, V., E. Bacaicoa, A.M.Zamarre˜no, E. Aguirre, M.Garnica, M. Fuentes and J.M.García-Mina. 2010. Action of humic acid on promotion of cucumber shoot growth involves nitrate-related changes associated with the root-to-shoot distribution of cytokinins, polyamines and mineral nutrients. *J. Plant Physiol.* 167 (8): 633-642.

Muscolo, A., M. Felici, G. Concheri and S. Nardi. 1993. Effect of earth worm humic substances on esterase and per-oxidase activity during growth of leaf explants of *Nicotiana plumbaginifolia*. *Biology and Fertility of Soils* 15: 127-131.

Muscolo, A., S. Cutrupi and S. Nardi. 1998. IAA detection in humic substances. Soil Biol. *Biochem*. 30 (8/9): 1199 – 201.

Muscolo, A.,F. Bovalo, F. Gionfriddo and S. Nardi. 1999. Earthworm humic matter produces auxin-like effects on *Daucus carota* cell growth and nitrate metabolism. *Soil Biology and Bio-chemistry.* 31: 1303-1311.

Nardi, S., G. Concheri, G. Dell’Agnola and P.Scrimin. 1991. Nitrate uptake and ATPase activity in oat seedlings in the presence of two humic fractions. *Soil Biology and Biochemistry* 23: 833-836.

Nardi, S., G. Concheri and G.Dell’Agnola. 1996. Biological activity of humic substances. In: Piccolo, A., (Ed.), Humic substances in terrestrial ecosystems. Elsevier, Amsterdam. p. 361-406

Nardi, S., D. Pizzeghello, A. Muscolo, F. DallaVecchia and G.Concheri. 1998. Effects of forest humus on biological activity in roots of *Pinus sylvestris* related to chemical humus fraction characterristics. *Fresenius Environ-metal Bulletin* 7: 203-208.

Nardi, S., D. Pizzeghello, A. Muscolo and A. Vianello. 2002. Physiological effects of humic substances on higher plants. *Soil Biology and Biochemistry* 34: 1527-1536.

O’Donnell, R.W. 1973. The auxin-like effects of humic preparations from leonardite. *Soil Sci.* 116: 106-112.

Phuong, H.K. and V. Tichy.1976. Activity of humus acids from peat as studied by means of some growth regulator bioassays. *Biologia Plantarum*18: 195-199.

Rerabek, J. 1963. The relation of humic acids to the inhibition of plant straight growth. *Naturwissenschaften*50: 309-310.

SAS Institute Inc. 2002. Cary, NC, USA.

Sembawa Research Centre. 2008. *Road Map: Processing and Marketing of Rubber.*

Sembawa Research Centre and Plantation Agency of South Sumatera Province. Palembang.

Tan, K.H. and D. Tantiwiramanond. 1983. Effect of humic acids on nodulation and dry matter production of soybean, peanut, and clover. Soil Science Society of American Journal. 47: 1121-1124.

Tattini, M., P. Bertoni, A. Landi and M.L. Traversim. 1991. Effect of humic acids on growth and biomass partitioning of container grown olive plants. Acta Horticulturae. 294: 75-80.

Trevisan, S., A. Botton, S. Vaccaro, A. Vezzaro, S. Quaggiotti and S. Nardi. 2011. Humic substances affect *Arabidopsis* physiology by altering the expression of genes involved in primary metabolism, growth and development. Environ-mental and Experimental Botany. 74: 45-55.

Vaccaro, S., A. Muscolo, D. Pizzeghello, R. Spaccini, A. Piccolo and S.Nardi. 2009. Effect of a compost and its water-soluble fractions on key enzymes of nitrogen metabolism in maize seedlings. J. Agric. Food Chem. 57 (23): 11267-11276.

Valdrighi, M.M., A. Pera, M. Agnolucci, S. Frassinetti, D. Lunardi and G. Vallini. 1996. Effects of compost-derived humic acids on vegetable biomass production and microbial growth within a plant (*Cichorium intybus*)-soil sys-tem: a comparative study. Agriculture, Eco-systems and Environment. 58:133-144.

Vaughan, D. and R.E. Malcolm. 1985. Influence of humic substances on growth and physiological processes. In: Vaughan, D. andR.E. Malcolm (Eds.). Soil organic matter and biological activity, Martinus Nijhoff/Junk W, Dordrecht, The Netherlands. p. 37-76.

Varanini, Z. and R. Pinton. 1995. Humic substances and plant nutrition. In: Lüttge, U., (Ed.), Progress in Botany, Springer. Berlin. 56: 97-117.

Varanini, Z. and R. Pinton. 2001. In: Pinton, R., Z. Varanini and P. Nannipieri (Eds.), Direct versus indirect effects of soil humic substances on plant growth and nutrition.The Rhizosphere, Basel. p. 141-158.

Visser, S.A. 1986. Effetto delle sostanze umiche sulla crescita delle piante. in: Burns, R.G., G. Dell’Agnola, S. Miele, S. Nardi, G. Savoini, M. Schnitzer, P. Sequi, D. Vaughan and S.A.Visser (Eds.), Sostanze Umiche. Effettisul Terreno e sulle Piante, Ramo Editorial edegli Agricoltori. Roma. p. 96-143.