EFFECT OF LIGNOHUMATE ON YIELD AND QUALITY OF RICE IN A PADDY FIELD IN BALI, INDONESIA

ВЛИЯНИЕ ГУМИНОВОГО ПРЕПАРАТА ЛИГНОГУМАТ НА УРОЖАЙНОСТЬ И КАЧЕСТВО ЗЕРНА РИСА В УСЛОВИЯХ ЗАТОПЛЯЕМОГО КУЛЬТИВИРОВАНИЯ НА О. БАЛИ, ИНДОНЕЗИЯ

Organic matter is needed in the soil as an essential component for good plant growth. While most of the paddy field in Bali is a shortage of organic matter so that the productivity of land for rice is very low. Therefore the effects of commercial humic product Lignohumate AM® (**LH**) on plant growth, yield, and grain quality were evaluated in a field trial using traditional wet rice (*Oryza sativa* L.) cultivation in Bali, Indonesia. The experimental design included insecticidal sprays with beta-cyfluthrinat 100, 50, and 0% of recommended application rates, either alone or in combination with LH. The LH was also applied for seed pre-treatment before sowing. For non-LH treatments, equal amounts of water were used. Plant growth variables and substances contents of the treated rice were recorded. The data were variant analysed according to a randomized complete design and the differences among treatments were compared by Duncan Multiple Range test at levels of 1% and 5%. No significant effects on plant height, number of tillers, leaf number, leaf area or rice yield were recorded. LH stimulated root growth at early stages of plant development, promoted chlorophyll synthesis in leaves, and increased 1000-grain weight and number of grains per hill. Due to the increased level of chlorophyll, LH application promoted an increase in carbohydrate levels and amylose content in rice. The experiment showed that when using LH, rice can be grown at significantly lower doses of pesticides than is currently used.

Key words: rice cultivation, humic product, grain quality

Большая часть сельскохозяйственных угодий острова Бали характеризуется дефицитом органического вещества, низкой продуктивностью почвы и высоким остаточным содержанием пестицидов. Повсеместное применение пестицидов сильно загрязняет поверхностные воды острова. Гуминовые препараты способны оказывать стимулирующее воздействие на рост и устойчивость растений, позволяя снизить дозы химических средств защиты за счет синергетического эффекта с пестицидами.

В полевом опыте в условиях о. Бали (Индонезия) изучено влияние гуминового препарата Лигногумат АМ® (**ЛГ**) на рост растений, урожайность и качество зерна при традиционном затопляемом способе возделывания риса (*Oryza sativa* L.). Схема опыта включала листовую обработку инсектицидом Бета-цифлутрин в дозах 100, 50 и 0% от рекомендуемых, как отдельно, так и в комбинации с ЛГ. В вариантах с внесением ЛГ проводили предпосевную обработку семян и опрыскивание по вегетации 0,05%-раствором ЛГ совместно с пестицидом.

Обработка ЛГ стимулировала рост корней на ранних стадиях развития растений, способствовала синтезу хлорофилла в листьях и достоверно увеличивала массу 1000 зерен и количество зерен в колосе. Существенного воздействия на высоту растений, число побегов, количество и площадь листьев, и урожайность риса не обнаружено. Повышение уровня хлорофилла при применении ЛГ способствовало увеличению содержания углеводов в зерне риса.

Эксперимент показал, что при использовании ЛГ для возделывания риса можно применять значительно меньшие дозы пестицидов, чем в настоящее время, поскольку при 50%-ной дозе пестицида урожайность и показатели качества зерна были не хуже, чем при традиционной технологии выращивания.

Ключевые слова: гуминовые продукты, пестициды, возделывание риса

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1. Introduction

Increasing population requires increasing food productivity especially rice since it of staple food for most people in the world. Many efforts have been done including improvement on culture practices such as fertilization, pest control, even genetic raising, however, all still needed the work of some hard working to fulfil the lack of the need. Ones should be done is completing the nutrient availability for rice to increase the productivity. Rice cultivation in tropical areas requires a significant amount of fertilizers and application of advanced agricultural technics.

In Bali agriculture contributes a big part of national economy. A special system of wet rice agriculture called Subak, involving local religions and traditions, is being used in Bali since thousand years ago. In these traditions, the relationships between human beings and the environment is one of the top priorities. In this context, the negative consequences of using high amounts of fertilizers even obtain higher importance, not only environmental, but social as well. Thus, mineral fertilizers leach downhill, contaminate waters, and finally they are costly. Organic fertilizers, recommended by local government (Petroganik), including farm manure and composts from manures (cow dung, goat, poultry, etc.), industrial wastes (waste sugar mill), or municipal waste (household waste) filler, are not enough [1].

An alternative or a supplemental tool can be using of humic substances (**HS**): natural materials, which have been formed from degraded biomass. HS are chemically defined as organic macromolecular complexes that contain phenolic, carboxylic, and aliphatic moieties, acting as colloidal component, having high surface area and ion absorption capacity [2]. HS affect plant physiology [3, 4], enhance nutrient uptake [5-7], raise the phytochemical decomposition of pesticide or toxic organic compounds [8], and play a role in transformation, binding and transport of toxic chemicals in soil and water [9]. Besides, HS have been reported to exert antitoxic actions in contaminated environment [10, 11], binding pesticides and providing mediating effects [12]. Pesticide toxicity in the presence of HS was examined in numerous studies [13-15] and showed, that they can either enhance or reduce toxicity of xenobiotics, exerting synergistic or antagonistic effects on plant growth, depending on chemical and physiological mechanisms involved [10, 11, 16, 17].

Therefore, application of HS-based amendments (or commercial humic products – **HP**) as fertilizers or plant growth stimulators in agriculture has increased in recent years [18-26]. HP can be used not only as soil conditioners, but also as plant biostimulants [7, 27]. In latter case they can be applied as foliar sprays or solutions for seed pre-treatment before sowing. Positive experience of HP-applications as foliar treatments in greenhouses or field studies has been reported not only to increase yield, but also to improve crop quality [28-30].

In this study we assessed the effects of commercial HP Lignohumate®, which has been derived from lignocellulose wastes [31, 32]. It is manufactured in a controlled process of hydrolytic oxidation of lignosulphonate, resulting in formation of humic-like substances. Lignohumate has been profoundly analyzed and widely tested on various crops and environmental conditions. The results have demonstrated its effectivity as a plant growth stimulator, providing an increase in yield and synergistic effect with pesticides [33, 34].

The objective of this study was to evaluate the effect of Lignohumate on growth, development and yield on rice *Oryza sativa* L. both along and at combined application with pesticide under conditions of traditional wet rice cultivation in Bali.

2. Materials and Methods

2.1. Research Site Condition

The research was conducted in technical irrigated rice field of Subak Anggabaya (Subak is irrigation system in Bali that has own traditional rule that convincing the balance of water sharing to fulfil the water need) Bali, Indonesia during the dry season of 2015.The soil type of the experimental field (was analyzed by Soil Lab authority of Udayana University) is Latosol with properties (at depth of 0–20 cm) as follows:17.32% sand, 25.23% clay, 0.92% organic carbon, 0.2% total nitrogen, 31.97 ppm total phosphorus, pH 7.0 and 0.83 cm min-1 conductivity.

2.2. Crop, Fertilizers, and Experimental Design

Rice (*Oryza sativa* L., HYV Cigeulis) was cultivated under fully irrigated conditions. Experimental design included basic NPK fertilization, insecticidal sprays with beta-cyfluthrin(active ingredient, **a.i.**) (Sumo 50ECTM), and the application of commercial humic product «Lignohumate AM» (**LH**), so that treatments composed two blocks: with application of LH (LH-treatments, **LHT**) and without (non-lignohumate treatments, **NLHT**).Before sowing, rice seeds were soaked in water for NLHT or in 0.5% LH solution for LHT. After 2 weeks, seedlings were transplanted on seed bed, the period from sowing until harvesting was 95 days.

The experiment was laid out in complete randomized design with 4 replications of 4 m x 5 m blocks and planting spacing of 30 cm x30 cm.All the plots were treated with basic NPK fertilization: Urea (46% N) and Ponska (15% N, 15% P_2O_5 and 15% K_2O) with doses of Urea 2 kg/100 m^2 + Ponska 2 kg/100 m^2 which distributed three times applications as follows: 1) one week after transplanting: 0.5 kg urea/100 m^2+ 0.5 kg Ponska/100 m^2, 2) three weeks after transplanting: 0.75 kg urea/100 m^2 + 0.75 kg Phonska/100 m^2, and 3) five weeks after transplanting: 0.75 kg urea/100 m^2 + 0.75 kg Phonska/100 m^2.Beta-cyfluthrin was applied as foliar spray at 2 and 8 weeks after transplanting (together with LH application) at concentrations of 1.0, 0.5 and 0 mL L^{-1}, which correspond to 100%, 50% and 0% of the recommended dose. Reduced concentrations of insecticide were used to evaluate a possible synergetic effect at the time of application in combination with humic product. «Lignohumate AM» is a commercial humic product with microelements, derived by an industrial process from lignin and containing about 80% of humic substances, 9% K, 3% S, 0.1-1.2 g kg^{-1} Mg, 0.1-2.0 g kg^{-1} Fe, 0.1-1.2 g kg^{-1} Cu and Co, 0.1-1.5 g kg^{-1} B, 0.05-1.15 g kg^{-1} Mo, 0.1-1.2 g kg^{-1} Zn (on dry matter basis).LH was applied for seed pre-treatment before sowing (soaking in 0.5% LH solution) and as foliar spray (0.05%) in a tank mixture with insecticide at 2 and 8 weeks after transplanting, which corresponded to growth stages of tilling and booting. Field trial design is summarized in *Tab. 1*.

Table 1
Design of the field trial treatments

Treatment*	Insecticide, mL L^{-1} (% ofrecommended rate)	Lignohumate
A	1.0 (100)	no
B	0.5 (50)	no
C	0 (0)	no
D	1.0 (100)	yes
E	0.5 (50)	yes
F	0 (0)	yes

*Basic NPK fertilization is equal for all the treatments.

2.3. Data Collection and Analysis

During 1st five weeks seedlings were sampled weekly to record growth characters: a random sample of 2 plants from each plot was taken to the laboratory and analyzed for plant height, leaf area, tiller and leaf number per hill, and chlorophyll content in leaves. Chlorophyll content was measured using Chlorophyll Meter type SPAD-502Plus (Konica Minolta) with its unit is SPAD.At maturity the crop was harvested manually.After threshing, cleaning and drying the grain and straw, grain fresh weight and oven weight, 1000-grain dry and oven weight, numbers of seed and panicles per hill were recorded. To determine yield and yield components random samples of 20 hills were taken from each experimental plot and analyzed.

2.3.1. Chemical analysis of grain

Ash content was determined using thermogravimetric SNI 01-1891-1992. Total nitrogen was analyzed by modified of semi micro Kjeldahl AOAC 960.52; protein content was calculated from total nitrogen content with conversion rate of 6.25 [35].Lipids were analyzed by extraction series of diethyl ether, n-hexane, petroleum ether and benzene; the percentage of dry lipids was calculated after evaporation of extracts.Total carbohydrate percentage was determined by difference i.e. resulted from 100 subtracted by percentage of sample water content, ash, protein and lipid. Amylose was analyzed using colorimetric iodine assay index method.The amylose content calculated by adjusting absorbance (λ 625 nm of spectrophotometer) of sample to standard curve constructed using pure amylose [36, 37].

2.3.2. Statistics

The data were variant analyzed according to the randomized complete design using the Microsoft Excel software. When the difference between the treatments was significant, this difference was compared by Duncan Multiple Range Test on level of 1 and 5% [38].

3. Results

3.1. Plant Growth

At early stages of plant vegetation LH promoted root growth of seedlings: the height of seedling was significantly different between NLHT and LHT according to 5% degrees using the Duncan Multiple Range test (*Tab. 2*). At 2 weeks after sowing the average root length of LH-treatments was significantly longer than on NLHT (13.1 and 12.3 cm respectively), while seedling height was lower than on NLHT (19.4 and 20.4 cm). The LH-treatment reveal fatter seedling but shorter than NLHT, so they looked more vigorous, more robust, and its stems was thicker than without LH application (*Tab. 2, Fig. 1*).

With subsequent plant growth and development, the difference in height between LHT and NLHT was less significant (*Tab. 3*). The others variables such as total number of tillers, including the number of productive tillers per hill showed no significant differences among treatments (*Tab. 3*). Hence, by the 5th week of vegetation plant height, numbers of tillers and leaf per hill in LHT slightly exceeded the corresponding parameters in NLHT (*Fig. 2*).

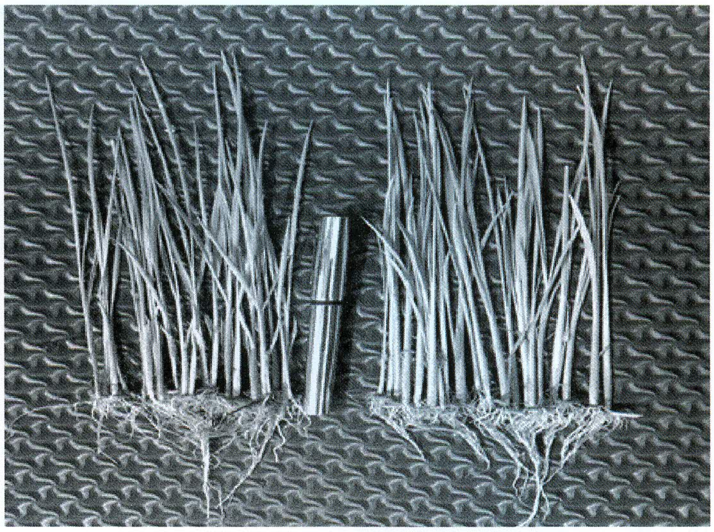

Fig. 1. Seedling of 10 days after sowing on NLHT (left) and LHT (right). Seedlings with LHT looked more vigor than NLHT.

Table 3
Plant height, tiller number, and productive tiller number 3 weeks after transplanting

Treatments	Plant height (cm)	Total tiller hill^{-1} (No.)	Productive tillers hill^{-1} (No.)
A	60.8±0.2 a	27.4±0.4 a	16.6±2.3 a
B	59.3±1.1 a	25.0±0.9 a	17.4±2.2 a
C	54.9±0.8 ab	25.8±0.1 a	19.1±2.4 a
D	57.3±0.5 ab	21.6±2.0 a	18.7±3.3 a
E	57.2±0.2 ab	23.8±1.2 a	19.3±4.2 a
F	52.2±1.1 b	24.1±1.2 a	17.4±0.5 a
NLHT	60.8±0.2 a	27.4±0.9 a	16.6±0.6 a
LHT	59.3±3.0 a	25.0±0.6 a	17.4±0.3 a

Description: Data are means±SDs. Numbers followed by the same letters are not significantly different based on 5% Duncan test level (above data block). Numbers followed by the same letters are not significantly different based on 5% Student t-test (below data block).

Table 2
Seedling height and root length at 2 weeks after sowing

	NLHT[1]		LHT[2]	
	Root	Shoot	Root	Shoot
Length (cm)	12.3±0.4	20.1±0.7	13.1±0.3	19.4±0.2
Signification. Student t-test 5% (n=45)	A t (calculated) = 8.95 t (table) = 1.98	a t (calculated) = 13.12 t (table) = 2.18	B	b

[1]Non-lignohumate-treatments, [2]Lignohumate-treatments.

Table 4
Leaf number, leaf area and chlorophyll content in rice leaves 5 weeks after transplanting

Treatments	Leaf number (No.)	Leaf area (cm² hill⁻¹)	Chlorophyll (SPAD unit)
A	71±3.0 a	8635±16 a	38.64±11 bc
B	69±6.0 a	9947±23 a	38.93±13 bc
C	63±7.0 a	8555±12 a	40.06±12 ac
D	60±0.0 a	8902±13 a	39.56±06 ac
E	69±0.1 a	8937±11 a	43.34±2.0 a
F	60±0.9 a	8640±12 a	40.84±4.0 ac
NLHT	67.7±3.5 a	9045±22 a	39.21±11 a
LHT	63.0±2.3 a	8826±23 a	41.25±12 b

Description: Numbers followed by the same letters are not significantly differentiated based on 5% Duncan test level (above data block). Numbers followed by the same letters are not differentiated based on 5% Student t-test (below data block).

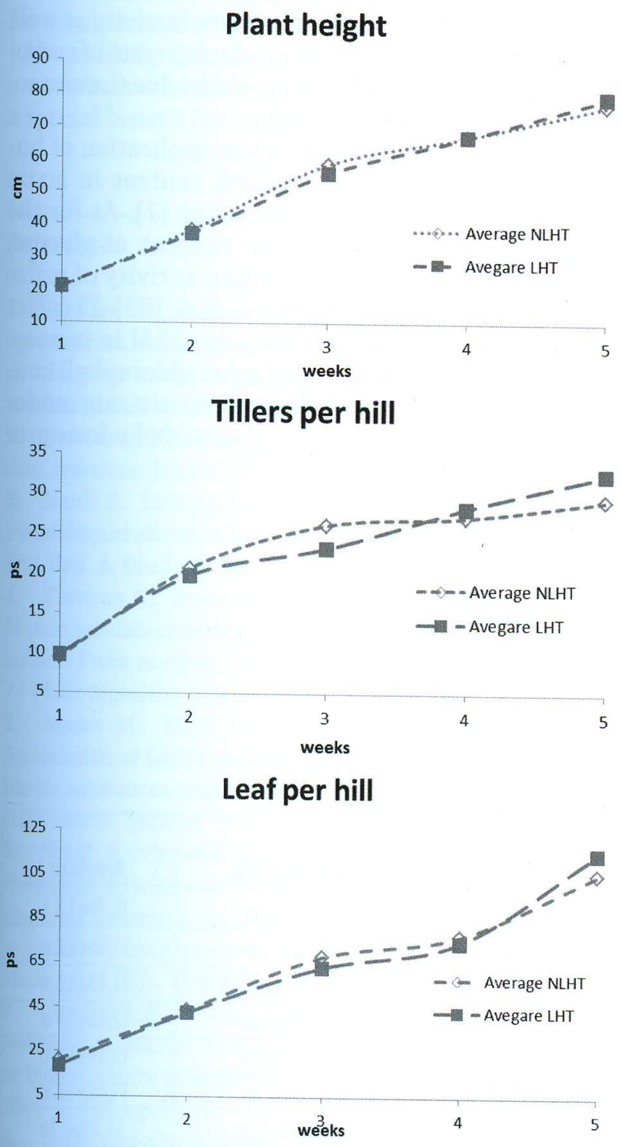

Fig. 2. Rice plants growth characteristics during 1st five weeks of vegetation. NLHT is non-lignohumate treatment; LHT is lignohumate treatment.

Table 5
Effect of LH and pesticide on rice yield and yield components

Treatments	1000-grain dry weight (g)	Dry yield (ton ha⁻¹)	Grain hill⁻¹ (No.)	Panicle hill⁻¹ (No.)
A	25.5±3.0 b	9.6±0.4 b	1412±12 a	27.4±1.6 a
B	24.7±3.3 b	11.0±0.3 ab	1571±19 a	25.0±2.3 a
C	25.4±4.0 b	9.9±0.4 ab	1805±13 a	25.8±1.3 a
D	24.6±3.0 b	10.4±0.2 ab	1709±23 a	21.6±2.5 a
E	36.5±3.3 a	11.5±0.3 a	1762±16 a	23.8±3.3 a
F	24.1±3.0 b	9.6±0.5 b	1410±11 a	24.1±4.5 a
NLHT	25.2±3.4 a	10.2±0.8 a	1596±15 a	26.1±3.4 a
LHT	28.4±5.3 b	10.5±1.1 a	1627±17 b	23.1±3.4 b

Description: Data are means±SDs. Numbers followed by the same letters are not significantly differentiated based on 5% Duncan test level (above data block). Numbers followed by the same letters are not differentiated based on 5% Student t-test (below data block).

The best effect of LH on chlorophyll concentration was in combination with 50% of recommended insecticide dose (i.e., 0.5 mL L^{-1} of beta-cyfluthrin a.i.). Total chlorophyll content on this treatment (E) was 43.34 SPAD which was significantly different to 100% insecticide dose with no LH involved (A) that was 38.64 SPAD. This means that LH was able to substitute 50% insecticide of the amount used by farmer. This is one benefit of applying organic product in agriculture that subsequently supports organic farming system. The high chlorophyll concentration increases photosynthesis activities of leaves and the product fulfil seed fully therefore seed set more pithy, hence contribute to better seed quality. *Tab. 4* shows LHT presented the average of chlorophyll concentration of 41.25 SPAD and was significantly higher than in NLHT that amount of 39.21 SPAD. Thus, increase in chlorophyll content was 5-12%. This percentage is related to rice yield that also increase significantly on E compared to A treatment (*Tab. 5*).

3.2. Rice Yield and Grain Quality

In general 1000-grain dry weight on LHT-plots was significantly higher compared to NLHT with an increase at about 13% (*Tab. 5*). Grain numbers per hill was also higher, but due to a decrease in the panicle number per hill, an increase in the harvested yield was insignificant.

The highest values for 1000-grain weight and harvested yield were observed for the treatment, where LHT was combined with 50%-dose of insecticide (36.5g and 11.5 t/ha; treatment E), which was significantly higher compared to 100% insecticide (25.5g and 9.6 t/ha) and to LHT alone.

Application of LH did not affect content of water, ash and protein in rice grain. But content of lipids was significantly reduced. The most remarkable differences were observed for the carbohydrate content: LH alone was able to raise carbohydrate and amylose concentration of rice. Treatment of 100% pesticide combined with LH produced the highest concentration of carbohydrate and amylose rice grain, they were 71.68 mg and 12.66 mg per 100 g rice respectively (treatment D; *Tab. 6*).

4. Discussion

Root growth stimulation resulting from application of HS is often reported in literature [39- 42]. Thus, application of HA in nutrient media in optimal concentrations promoted development of lateral roots, radicular superficial area, and number of mitotic sites in rice seedlings, acting as a plant elicitor by triggering a series of cell signalling events [43]. HA even has indicated more beneficial impact to root than auxins on the development of root system [44]. Similar effects have been reported earlier for the LH, when it was applied to maize plants and promoted an increase in root dry weight up to 117-124% and slight decrease of leaf dry weight (90-99% to control) [33].

LH was able to promote chlorophyll synthesis in plants. Similar phenomena, when HS applied as foliar treatment, improved photosynthetic rate due to the higher content of chlorophyll were reported for pepper [29], chrysanthemum [45]. Positive influence of humic product on photosynthetic parameters was also observed by Haider et al [46]. For LH an increase in chlorophyll content (up to 17-70%), was documented earlier [33, 34], suggesting its positive role in photosynthesis process.

The highest chlorophyll production on the treatment of 50%-rate of the insecticide combined with LH (treatment E) may have been caused by the combined effect of beta-cyfluthrin in combination with LH in the proper dosage, which provided maximum results. When 100%-rate of insecticide was applied, probably the insecticide exerted certain toxicity for plants, which influenced chlorophyll formation and resulted in its lower content in leaf, than in treatments with 50%-rate of insecticide or without it.

The highest values for 1000-grain weight and harvested yield were also documented for the treatment E, where LHT was combined with 50%-dose of insecticide. This means that LH is able to substitute 50% insecticide of the amount used by farmer which also indicated the benefits functions of LH. These data suggest that LH promoted formation of filled grains compared to NLHT, most probably due to its positive influence on chlorophyll synthesis. The chlorophyll is responsible for carbohydrate production through photosynthesis [47]. Increased chlorophyll content in the leaf leads to increase in the plant productivity as well, and the observed higher production rate of carbohydrates in LHT (Tab. 6) could be due to more active photosynthesis process.

Similar observations, when application of humic product enhanced starch content in maize grain were reported in literature [7]. As for the LH, its ability to increase content of glucose, fructose and rubisco enzymatic activity of maize plants was showed by Ertani et al. [33]. This fact was strongly linked to the role of LH in increasing leaf chlorophyll. The higher chlorophyll concentration promote photosynthesis rate under sun light and results on higher carbohydrate production [48, 49].

Table 6
The nutritional content of rice

Treatments	Content (mg/100g)					
	Water content	Ash	Protein	Lipid	Carbohydrate	Amylose
A	11,65±2,0 b	1,29±0,4 e	12,87±0,3 a	3,70±0,2 b	70,51±4,0 e	10,76±1,3 f
B	10,72±1,4 f	1,55±0,1 c	12,88±0,7 a	3,93±0,5 a	70,94±3,1 c	11,96±2,2 c
C	11,31±0,5 d	2,16±0,2 b	12,86±0,4 a	3,52±0,7 c	70,15±5,3 f	11,13±3,0 e
D	10,88±0,4 e	2,21±0,7 a	12,85±0,8 a	2,37±0,2 e	71,68±2,4 a	12,66±2,1 a
E	11,47±1,4 c	1,16±0,4 f	12,87±0,5 a	3,92±0,1 a	70,58±7,2 d	12,49±1,7 b
F	11,75±0,7 a	1,33±0,2 d	12,88±0,5 a	2,52±0,2 d	71,54±3,2 b	11,42±1,6 d
NLHT	11,23±0,3 a	1,67±0,2 a	12,87±0,5 a	3,72±0,5 a	70,53±4,1 a	11,28±2,1a
LHT	11,37±0,8 a	1,57±0,4 a	12,87±0,6a	2,94±0,2 b	71,27±4,2 b	12,19±1,8 b

Description: Numbers followed by the same letters are not significantly different based on 5% Duncan test level (above data block). Numbers followed by the same letters are not differentiated based on 5% Student t-test (below data block).

5. Conclusions

In this study commercial humic product Lignohumate AM® was for the first time tested for wet rice cultivation under conditions of tropics. Although no significant effects on plant height, number of tillers, leaf number, leaf area and rice yield were recorded, but some promising positive results were observed. Thus, LH stimulated root growth at early stages of plant development, promoted chlorophyll synthesis in leaves, and increased 1000-grain weight as well as the number of grains per hill. The experiment showed that when using LH, rice can be grown at significantly lower doses of pesticides than is currently used. Due to increase in chlorophyll, application of LH promoted an increase in carbohydrate levels and amylose content in rice, so that the grains become pithier.

The most distinct positive effects were observed for combination of LH with half-dose insecticide. LH was likely able to maintain plant resistance to a toxic influence of pesticide. This effect can be of a special benefit for farmers.

The results of this study show that the application of Lignohumate for wet rice cultivation under conditions of Bali can be promising. However further experiments need to be focused on optimization of its application under local climate conditions and agricultural practices. Experiments need also to be done on a variety of plants including vegetables and horticultural crops in order to get the optimum dosage of each plant.

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