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Research Article

Physiological Response and Yield of Pepper Vines (*Piper nigrum* L.) to Organic Pepper Foliar Fertilization

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Abstract

Experiments were conducted to study the physiological effect of the plant growth and its impact of the yield of pepper vine (*Piper nigrum*). The research was carried out on two varieties: Kuching and Semongok Aman-intended for production of black pepper for the first 30 months after planting. For the purpose of the experiment a foliar fertilizer, produced at the laboratory scale was used. The treatment consisted of T1: Chemical fertilizer, T2: foliar fertilizer, T3: Integrated fertilizer and T4: Control. The integrated fertilizer treatment out-yielded foliar fertilizer and chemical fertilizer by 72% and 15% respectively, with the higher yield being associated with various phenotypical alterations. It influences the growth rate of the shoot, stems and affects the formation of the foliage. The integrated fertilizer regime also has a positive effect upon the functional activity of photosynthetic pigments, improve leaf gas exchange and photosynthesis rate. Result also showed the high fertility level in foliar fertilizer regime reflected the importance of organic fertilizer in improving soil quality. However, the low production in solely organic foliar fertilizer regime also reflected the importance to add insufficient nutrient to sustain organic production. In conclusion, the integrated fertilization regime gave a significant increase in growth and yield of black pepper.

Keywords: Black Pepper; Foliar Fertilizer; Growth Rate; Yield

Introduction

In the past few years, it has been reported that the intensive agriculture aiming at high yields has led to a breach in the ecological equilibrium in the agricultural ecosystems [1-3]. To overcome this scenario, the development of new ecologically consistent technologies was imposed which would guarantee the steady growth of agriculture [4-6].

With an increase of quality conscious among end-users on pepper and its derivative product, almost all pepper producing countries have introduced an entirely new type of production technology which does not cause harm to the environment. Most often this new type of technology (organic agriculture) is defined as a system for maintenance of the natural fertility of the soil, biological diversity of the species and the ecological balance of the environment. This type of agriculture avoids or totally excludes the use of synthetic fertilizers, pesticides, growth regulators and food additives. It counts mostly on the role of the sowing alternations, the use of plant waste, manure and green fertilization, as well as the aspects of biological protection. A number of authors consider that organic production gives lower yields [7,8], however at the same time the harvest is characterized by better taste qualities compared to the one obtained through conventional production [9-11].

Several studies have proved the capability of foliar fertilizer in promoting the growth of crops such as canola, soybean, lentil, pea, wheat and radish [12,13]. Looking into this perspective, the farmers has to look for an alternative measures to sustain his farming business profitability. The utilization of seaweed and fish emulsion based liquid fertilizers are one of the prime inputs that stimulate plant growth as it has been well known as plant growth regulator containing various type of phytohormones and nutrients.

Bio-fertilizers obtained as a result of composting of fish emulsion with seaweed, have a number of advantages compared to the manure - they are richer in nutritive macro- and microelements, vitamins, enzymes, hormones, etc [14]. It was found that this foliar fertilizer increase the populations of the useful microorganisms

[15,16] which for their part synthesize biologically active substances. Bio-fertilizers not only improves soil fertility with their content of growth regulators but also have a stimulating effect upon plant growth and development [17-20].

In many countries the production and sale of bio-fertilizers in agriculture is well organized. The considerable interest towards them ensues from the fact that by influencing the soil fertility, they are also a naturally consistent solution of the problem for preservation of the environment and prevention of the plant production from contamination. The practical application of this type of bio-fertilizers has not been supported enough by comprehensive scientific information. This exactly motivates our research work. Therefore, the objective of this project is to determine the efficacy and efficiency of the newly developed pepper foliar fertilizer on pepper growth and production.

Materials and Methods

The experiment was carried out at a farmer's land in Kampung Niyor, Johor on soil of Munchong Series during the period of 2013 to 2017. The particle size analysis showed that the brownish yellow soil belongs to clay loam of fine sandy clay and had a pH of 5.74 (Table 1). Geographical location of the experimental site is 102009.549 Latitude and 02017.235 E Longitude. The average altitude of the site is 23.21 m above sea level, the mean temperature ranged between 27-33^{o}C, a mean annual rainfall of 1,500mm.

Chemical Properties	Soil depth (0-30cm)
pH	5.74
CEC (cmol (+)/kg	7.6
Exchangeable K^+ (cmol (+)/kg	0.74
Exchangeable Mg^{2+} (cmol (+)/kg	0.89
Exchangeable Ca^{2+} (cmol (+)/kg	0.98
Exchangeable Na^+ (cmol (+)/kg	0.18
Organic carbon (%)	1.49
C/N ratio	8.42
Total Nitrogen	0.41
Available phosphorus (mg/kg)	8.54
Total potassium (mg/kg)	24.08

Table 1: Initial chemical characteristics of soil at the experimental site.

Pepper vines of the variety Kuching were planted in rows with spacing of 2.1 m x 2.1 m between and within the rows, with a population of 2,000 vines per hectare. The experiment was carried out in a randomized complete block design (RCBD) with three replicates. Each replicate contained four treatments with 30 pepper vines per treatment. The four treatments consisted of:

- T1: NPK chemical fertilizer of formulation 12:12:17:2+TE, applied 6 times annually (at every 2-monthly intervals), following Malaysian Pepper Board's recommendation rate (Malaysian Pepper Board, 2011). The NPK chemical fertilizer was broadcasted evenly surrounding the mound, about 30-50 cm from the main stem of the pepper vine. In addition, ground magnesium limestone was applied once a year to neutralize the soil acidity.
- T2: Pepper foliar fertilizer application. The fertilizer was produced at MPB laboratory with the foliar fertilizer composition of seaweed extract with fish emulsion. Phytohormone and free amino acids analysis was performed prior to conducting the experiment. The assay shows that the foliar fertilizer contains various types of free amino acid (Figure 1) and 6 types of phytohormone viz: cytokinin (tz1), Jasmonic acid (JA), Salicylic acid (SA), Abscisic acid (ABA), Oxo-phytodienoic acid (OPDA) and Indole-3-acetic acid (IAA) that could stimulate the plant growth (Figure 2A-F). The nutrient contents in foliar fertilizer are shown in Table 2. The pepper vines were applied with 3L ha^{-1} of foliar fertilizer at monthly interval. Application was done early in the morning using motorized sprayer. The application of foliar fertilizer was carried out on the whole pepper crop, including the leaves, stems, branches, flowers and fruits.
- T3: both NPK chemical fertilizer and organic pepper foliar fertilizer were applied
- T4: (Control): no fertilizer was applied.

The plant was allowed to grow for 30 months of age so as to record growth parameter. The research was carried out on two pepper varieties: Kuching and Semongok Aman – intendedfor production of black pepper for grading. At the completion of harvesting cycle, the yield and yield components, and the leaf area index were measured and recorded. The concentration of photosynthesis pigment chlorophyll, the photosynthesis rate and transpiration rate were measured in leaf samples taken 12 months after 1 harvesting cycle by using a portable photosynthesis system (PPS System, model TPS 200). These analyses were repeated for yield and growth parameters for the subsequent 2 production cycles. Data were subjected to Analysis of Variance (ANOVA) and the significant level was set at P≤0.05 and P≤ 0.01. Differences between traits means were assessed using Duncan's Multiple Range Test at P≤0.05 level.

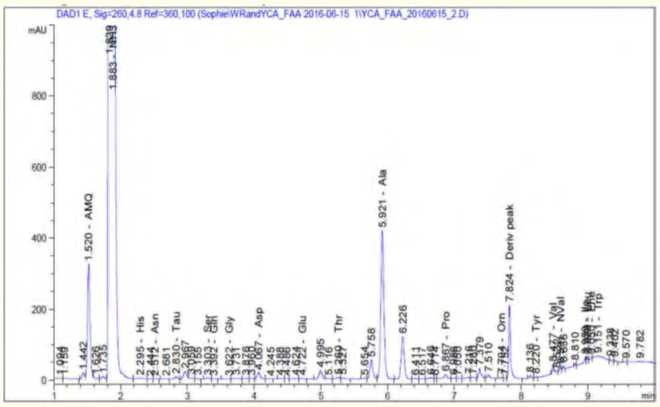

Figure 1: LCMS Chromatogram of free amino acids.

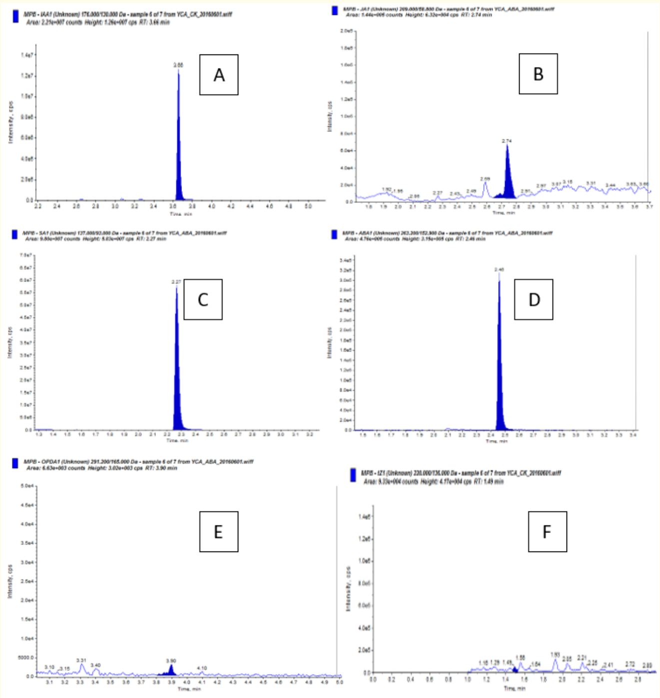

Figure 2: LCMS Chromatogram of Plant growth hormone. A: Indole-3-acetic acid, B: Jasmonic acid, C: Salicylic acid, D: Abscisic acid, E: Oxo-phytodienoic acid, F: Cytokinin

Nitrogen	0.45%	Boron	102 ppm
Phosphorus	0.31%	Cobalt	8.9 ppm
Potassium	3.2%	Jasmonic acid	0.036 pmol/µl
Magnesium	0.74%	Indole-3- acetic acid	0.048 pmol/µl
Calcium	1.02%	Salicylic acid	0.032 pmol/µl
Sulphur	0.85%	Cytokinin acid	0.012 pmol/µl

Table 2: Nutrient content of organic pepper foliar fertilizer.

Results and Discussion

The data (Table 3) show that nutrition with pepper foliar fertilizer influences plant growth. Results showed that all the fertilizer regimes were able to accelerate the growth rate of the roots and stems. The root length in pepper vines treated with both foliar fertilizer and chemical fertilizer is longer in both variety with the length of 95.7cm and 105.7 cm respectively. The similar growth character can also be detected in plant height and number of primaries on pepper vine. The tallest and the highest number of primaries were detected in integrated fertilizer regimes for both varieties. This was followed by the chemical fertilizer treatment. The application of pepper foliar fertilizer alone showed the shortest and lowest number of primaries in both varieties. The fresh root mass in both varieties is considerably higher as compared to the control variant. The better developed root system is a precondition for better nutrition of the pepper vines. The same tendency is observed with respect to the growth rate of the stem and leaf. These data correspond with the results obtained by other authors [21]. These finding also indicated that the foliar application could be attributed to the adequate nutrient supply which in turn improved all growth influencing characters. This might probably be due to the better utilization of nutrient supplied. In terms of growth characteristic among varieties, the variety Semongok Aman seems to be grown faster than Kuching variety. The finding was further elucidated by the formation of less empty node in SemongokAman than Kuching variety. Besides, the high root length detected in SemongokAman variety further strengthen the hypothesis that SemongokAman was more water resistant than Kuching variety.

Measurements were made 30 days after bio-fertilizer applied. Each data point represents the mean value of five plant samples + standard error. Values with an asterisk are statistically significant towards the control at 5% level, and values with two asterisks are statistically significant towards the control at 1% level.

Var.	Root length (cm)	Plant height (cm)	Number of primaries	Root dried weight (g)	Stem dried weight (g)	Leaf dried weight (g)	LAI
Kuching							
1	89.6 ± 11.2	209.5 ± 14.6	20.5+0.5	50.8 ± 2.6	339.8 ± 15.2	358.7 ± 11.5	5.2 ± 0.6
2	66.8 ± 10.9	109.5 ± 12.6	11.1+0.4	40.6 ± 3.8	153.6 ± 12.6	158.9 ± 15.4	2.6 ± 1.2
3	95.7 ± 10.8	220.0 ± 11.5	22.5 ± 0.6	58.7 ± 4.1	351.05 ± 11.5	375.5 ± 13.4	5.8 ± 0.8
4	40.5 ± 9.7	80.1 ± 12.5	9.5 ± 0.5	34.6 ± 3.5	126.8 ± 10.4	134.2 ± 15.0	1.8 ± 0.2
Semongok Aman							
1	99.6 ± 12.7	224.5 ± 16.3	24.5+5.2	62.1 ± 8.7	359.2 ± 25.7	378.4 ± 19.9	5.7 ± 0.9
2	54.8 ± 13.5	49.5 ± 14.9	10.1+4.7	43.3 ± 7.2	154.3 ± 22.1	178.1 ± 24.3	3.0 ± 0.8
3	105.7 ± 14.2	243.0 ± 16.5	23.5 ± 5.2	64.4 ± 9.1	371.7 ± 11.9	395.7 ± 22.7	6.3 ± 0.4
4	38.6 ± 15.2	34.6 ± 14.7	11.2 ± 4.5	37.5 ± 8.8	145.2 ± 21.0	153.6 ± 20.1	2.4 ± 0.2

Table 3: Influence of organic foliar fertilizer on growth of young pepper plants, var. Kuching and Var. SemongokAman; 1. Chemical fertilizer; 2. Pepper foliar fertilizer; 3. Integrated fertilizer; 4: Control

Total biomass productions are another useful qualitative method to estimate the vegetative growth of pepper under field condition. Total biomass increase with time irrespective of fertilizer treatment with the maximum plant dry weight being observed in integrated fertilizer regimes. The increase in plant dry weight was mainly due to the increased mass of the above and ground organs, e.g. leaves, stem, root, fruit spikes etc. Total dry matter accumulation from 0 to 30 months after planting exhibited a sigmoidal behavior like other annual crops, characterized by better growth rate in the period of leaf production and fruit formation (flowering to harvesting, 18-26 months after planting) with slow decline towards fruit maturation and harvest (Figure 3). The decline in dry matter can be explained by loss of plant parts like leaves and berries due to harvesting. The analysis also showed that the plot receiving foliar fertilizer have a better growth rate for the first 8 month after planting as compared to chemical treatment. The growth rate of this plot exhibit more or less similar plant dry matters to integrated fertilizer treatment indicated that foliar fertilizer play an important role in early plant growth. The explanation for this scenario is that at the early growth stage, particularly when pepper cutting was just transplanted to the field, the root system was not fully developed and the uptake of nutrient through foliar is therefore considered to be the perfect way to absorb the nutrient. Besides, the quantities of nutrient needed are small, and tolerances for the applied materials, and rates of uptake are adequate through foliar application. The results obtained also showed that pepper vine growth under integrated fertilizer regimes produce higher plant dry matter throughout the project. The high plant dry matter was mainly due to optimum nutrient uptake by pepper vines through soil and foliar based fertilizer especially during flowering and fruit development stage. The foliar fertilizer act as a supplement to the usual soil fertilizer treatment during flowering and fruit setting, when nutrient requirement are high but soil application would be too late or not physically practical. Even though the utilization of foliar fertilizer has been reported to enable and sustain crop growth and yield [22], but sole dependence on foliar fertilizer results in a lower plant biomass after 8 months onwards. This could be due to limited nutrient that the soil could supply with additional of a small quantity of external input. This finding was further confirmed by the research reported by Yap (2012) [23]. He reported that pepper vines required approximately 293-46-265 kg o N-P-K per hectare annually in order to sustain high yield and soil nutrient balance.

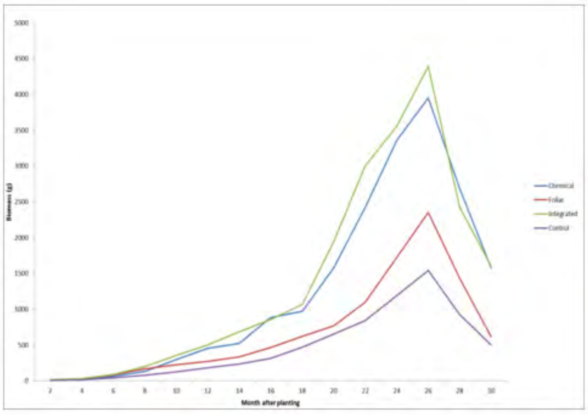

Figure 3: Effect of different fertilizer treatment on plant dry weight of pepper.

One of the main factors determining the quantity of absorbed sun energy and CO_2 from the pepper vines is the size of the photosynthesizing leaves. Data show that nutrition with integrated fertilizer regimes influences the leaf area index. The influence of the bio-fertilizer has been mathematically proven for both varieties and for both nutrition levels. The effect of different fertilizer regimes varies upon the content of photosynthetic pigments (Table 4). 30 days after the nutrition the content of chlorophyll a increases by 6% on average in Kuchingvariety and by8% in Semongok Aman variety when compared to normal culture practice by using chemical fertilizer. Under application of pepper foliar fertilizer, content of chlorophyll a was decrease to 4.46 from 2.86 for Kuching variety and to 1.51 under Semongok Aman variety. With respect to chlorophyll b and total chlorophyll contents, the same tendency is observed. The differences in chlorophyll content between the plants fed with bio-fertilizer and the control plants may result from the increased biosynthesis of the latter on the one part, and from the stabilizing effect of the bio-fertilizer upon the chlorophyll and protein complex.

Varieties	Chlorophyll "a"	Chlorophyll "b"	Total Chlorophyll (a+b)
Var. Kuching			
1	2.86 ± 0.5	1.29 ± 0.05	4.14 ± 0.5
2	1.46 ± 0.2	1.01 ± 0.03	2.47 ± 0.2
3	3.05 ± 0.3	1.52 ± 0.08	4.57 ± 0.3
4	0.83 ± 0.2	0.38 ± 0.02	1.21 ± 0.1
Var. Semongok Aman			
1	2.74 ± 0.1	1.26 ± 0.04	4.00 ± 0.2
2	1.51 ± 0.2	0.86 ± 0.01	2.37 ± 0.1
3	2.96 ± 0.2	1.82 ± 0.06	4.78 ± 0.1
4	0.76 ± 0.1	0.42 ± 0.04	1.18 ± 0.09

Table 4: Photosynthetic pigment content [mg g^{-1} fresh weight] in the fifth fully developed leaf of pepper vines var. Kuching and var. Semongok Aman after the application of the 1. Chemical fertilizer; 2. Pepper foliar fertilizer; 3. Integrated fertilizer. 4: Control.

Measurements were made 30 days after bio-fertilizer applied. Each data point represents the mean value of five plant samples + standard error. Values with an asterisk are statistically significant towards the control at 5% level, and values with two asterisks are statistically significant towards the control at 1% level.

No deviations from the standard in the ratios between the photosynthetic pigments are observed which shows that the separate pigments are equally influenced by the nutrition with the bio fertilizer. According to many authors [24,25] the improved leaf gas exchange is a precondition for higher productivity of the plants. Our research shows (Table 5) that usage of chemical fertilizer incorporated with foliar fertilizer application improves the leaf gas exchange. In Kuching variety, the speed of the net photosynthesis was the highest in integrated fertilizer regimes, followed by chemical fertilizer regimes and foliar fertilizer regime with the photosynthesis value of 27.42, 24.68 and 15.75 µmol m-2 s-1 which is higher compared to the control vines (12.05). Similarly, the same scenario also detected in Semongok Aman variety with the photosynthesis rate of 25.69,22.37 and 13.20 µmol m-2 s-1 in integrated, chemical and foliar fertilizer regime respectively. The intensity of the transpirations was not differing considerably among treatment for Kuchingvariety accepted between integrated fertilizer regimes. The same was also happen in Semongok Aman variety. At the same time the stomatal conductance in the pepper vines to which the chemical and integrated fertilizer regime were applied, is higher. The ratio of photosynthesis to transpiration (instantaneous water use efficacy) was accordingly higher in integrated fertilizer treatment for both varieties; with loss of one m mol of water, 3.83 and 2.08 and 5.26 µ mol and 3.56, 1.80 and 4.75 µ mol of CO_2 for Kuching and Semongok Aman variety respectively were fixed in pepper vine grown under chemical, foliar and integrated fertilizer treatments, respectively. A high photosynthetic rate with lower CO_2 -concentration inside the sub stomata cavity in integrated fertilizer treatment suggested a more efficient carboxylation system (Table

Varieties	P_N	E	g_s
Var. Kuching			
1A	24.68 ± 2.1	6.45 ± 0.6	0.18 ± 0.01
2B	15.75 ± 1.8	7.58 ± 0.5	0.13 ± 0.01
3C	27.42 ± 2.0	5.21 ± 0.3	0.17 ± 0.02
4D	12.05 ± .2	8.09 ± 0.6a	0.09 ± 0.01
Var. Semongok Aman			
1	22.37 ± 2.1	6.29 ± 0.6	0.14 ± 0.01
2	13.20 ± 0.9	7.34 ± 0.5	0.13 ± 0.01
3	25.69 ± 1.6	5.41 ± 0.6	0.16 ± 0.02
4	12.65 ± 0.7	8.15 ± 0.4	1.06 ± 0.02

Table 5: Leaf gas exchange in the fifth fully developed leaf of pepper vines var. Kuching and var. Semongok Aman after the application of the 1. Chemical fertilizer; 2. Pepper foliar fertilizert; 3. Integrated fertilizer.; 4: Control: PN – Net photosynthetic rate [µmol $m^{-2}s^{-1}$]; E– Transpiration rate [mmol $m^{-2}s^{-1}$]; gs – Stomatal conductance [mol $m^{-2}s^{-1}$].

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5). The instantaneous water use efficacy on the leaf (represented by the ratio of photosynthesis to transpiration) is a measurement of carbon gained through photosynthesis with per unit of water transpired. A higher phot synthetic rate with lower transpiration in integrated fertilizer treatment indicated that water was used more efficiently in this than in foliar fertilizer treatment.

The data for the increased speed of the photosynthesis are unidirectional with those related to the content of photosynthetic pigments. This shows that along with the stomatal conductance the increased pigment content is one of the reasons for the higher photosynthetic speed in the plants to which the foliar fertilizer is applied. Contradictory data about the relationship between photosynthetic intensity and chlorophyll content of leaves have been reported. Along with the reports for the lack of linear relation between two indices there are other reports that higher total chlorophyll content is one of the main factors stimulating the rate of photosynthesis and biological productivity of organisms [26]. Our results support this hypothesis.

One of the yield components which determine its quantity to a large extent is the total green berries produced. The results (Table 6) show that nutrition with the addition of foliar fertilizer to chemical fertilizer regimes had a positive effect on this index. The total green berries produce per vines basis was increased by 14% in Kuching variety and by 13% in Semongok Aman varierty when compared with normal cultural practices (Chemical treatment). Whereas, when the pepper were treated with pepper foliar fertilizer alone, the total green pepper yield was drop to about 50% as compared to chemical treatment. This might probably due to limited nutrient being supplied for the vines as the foliar nutrient only available during the time of application. However, when this foliar was used in combination with chemical fertilizer regimes, it seem to be influence the yield. The explanation for this scenario is that the maximum uptake of nutrients by pepper vines by root and foliar mechanism. Furthermore, a plant's entire requirement for many trace elements may not be supplied by normal chemical soil fertilizer applications and can only be supply through application to the above ground part. This is because the quantities needed are small, and tolerances for the applied materials, and rates of uptake are adequate. Results also showed that the black pepper yield was higher in both varieties when pepper vines were treated with both chemical and fertilizer with the yield of black pepper were 2.58kg and 2.95 kg respectively. The increased yield results from the increased mass as well as from the increased number of the fruits spike in plants to which the foliar fertilizer was applied.

Varieties	Number of fruit spikes	Total green berries/vines	Total black pepper/vines
Var. Kuching			
1	985.48 ± 14.6	7.14 ± 1.2	2.24 ± 0.4
2	572.60 ± 10.2	3.84 ± 0.9	1.21 ± 0.3
3	1157.36 ± 21.3	8.17 ± 1.5	2.58 ± 0.3
4	337.69 ± 11.6	2.19 ± 0.7	0.62 ± 0.1
Var. Semongok Aman			
1	946.4 ± 15.1	8.52 ± 1.2	2.52 ± 0.5
2	488.3 ± 9.5	3.59 ± 0.8	1.53 ± 0.6
3	972.6 ± 13.8	9.67 ± 1.3	2.95 ± 0.2
4	275.8 ± 8.4	2.31 ± 0.3	0.88 ± 0.1

Table 6: Pepper yield (raw material for drying) after nutrition with different fertilizer regimes:1. Chemical fertilizer; 2. Pepper foliar fertilizer; 3. Integrated fertilizer; 4: Control.

The most important specification in the black pepper for grading is the bulk density. The results show that nutrition with foliar fertilizer when used in combination with chemical fertilizer has a positive effect upon this index (Table 7). The application of pepper foliar fertilizer alone have significantly decreased the bulk density of the pepper and therefore affect its quality. Pepper treated with integrated fertilizer treatment produced the highest grade of pepper quality (Malaysian Black Pepper No.1) with the bulk density value of 553.21 and 564.48 in Kuching and Semongok Aman variety. On the other hand, the vines treated with pepper foliar fertilizer only manage to produce Sarawak Field Black with the bulk

Varieties	Bulk Density	Quality
Kuching		
1	535.50	Sarawak FAQ Black
2	455.26	Sarawak Field Black
3	553.21	Malaysian Black Pepper No.1
4	321.93	Sarawak Coarse Field Black
Semongok Aman		
1	557.56	Malaysian Black Pepper No.1
2	479.51	Sarawak Field Black
3	564.48	Malaysian Black Pepper No.1
4	345.15	Sarawak Coarse Field Black

Table 7: Bulk density of black pepper – var. Kuching 50 and var. Semongok Aman*:*

1. Chemical fertilizer; 2. Pepper foliar fertilizer; 3. Integrated fertilizer; 4: Control

density of 455.26 and 479.51. This indicated the nutrient supplied by pepper foliar fertilizer alone was insufficient to sustain pepper production, it must be used in combination with chemical fertilizer in order to boost up the pepper production and to benefit the overall industry as a whole.

Conclusion

On the basis of the obtained results it was found that nutrition with pepper foliar fertilizer can only increases the plant growth and production when used in combination with chemical fertilizer. The sole application of pepper fertilizer led to poor vegetative growth and reduces overall production of pepper for about 50%. Field trial showed that the integrated fertilizer regimes are the best option for proper nutrient management for pepper. The application of both foliar and chemical fertilizer influences the growth rate of the roots and stem of the plant, as well as the leaves formation. There is a positive effect of the pepper foliar fertilizer with chemical fertilizer upon the functional activity of the photosynthetic apparatus/ increased content of the photosynthetic pigments, improved leaf gas exchange/. The integrated fertilizer regimes also influence the yield and pepper quality. The latter increases by 13-14% in both Kuching and Semongok Aman variety by producing black pepper with bulk density above 500g/L.

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