



## Effects of relay intercropping model and application of biological agents on the growth and yield of hot pepper

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### Abstract

Tiyung cultivar, the consumer preferences of hot pepper in Magelang Regency, is still low in productivity (7 ton.ha<sup>-1</sup>). This research aimed to determine the growth and yield of hot pepper planted in relay intercropping as affected by biological agent application as a technology to increase productivity. This research was arranged in a Factorial Randomized Complete Block Design with an intercropping pattern of hot pepper (two and three rows model) as first factor. The second factor was the application of biological agents, consisting of *Trichoderma asperellum*, *Bacillus velezensis* B-27, and Arbuscular Mycorrhizal Fungi (with and without biological agents). The control was the monoculture of hot pepper without biological agent application. The data observed were analyzed using Analysis of Variance and followed by the Duncan's Multiple Range Test at  $\alpha=5\%$ . The results showed that the relay intercropping of hot pepper produced less numbers of branches and leaves. It indicated more efficiency in the use of assimilates for the development of generative organs, thereby resulting in the high values fruits weight per plant, which were as high as those in monoculture. The productivity observed in relay intercropping was 12.93 ton.ha<sup>-1</sup>, which was 15.8 % higher than in monoculture. The application of biological agents significantly increased the stomatal density, the size of stomatal opening, the number of leaves, weight of fruits per plant and productivity of hot pepper. The productivity of plant applied with biological agents was 16.84 ton.ha<sup>-1</sup>, which was 86.50 % higher than that without biological agents application.

## INTRODUCTION

Hot pepper (*Capsicum frutescens* L.) is a horticultural crop from Solanaceae family, which has not only high economic value but also a complete combination of color, taste and nutritional value (Kouassi et al., 2012). The need for hot pepper will continue to increase in line with the increase in population or demand from consumers. The need for hot pepper consumption was quite high in 2015, reaching 318.21 thousand tons (Yanuarti and Afsari, 2016). Hot pepper productivity in Indonesia is still low, 7.78 ton.ha<sup>-1</sup> (Badan Pusat Statistik, 2018), while it's potential

can reach 12 ton.ha<sup>-1</sup> to 20 ton.ha<sup>-1</sup> (Sujitno and Dianawati, 2015). The productivity of hot pepper in Magelang Regency, Central Java is 7 ton.ha<sup>-1</sup> (Direktorat Jenderal Hortikultura Kementerian Pertanian, 2016), which is still lower than national productivity.

Planting horticultural and food crops, such as Tiyung hot pepper and sweet corn is a common practice among farmers in Magelang Regency. Tiyung cultivar is popular among farmers because it is resistant to yellow leaf curl disease. This common practice is called food diversification program as an effort to achieve food security. Relay intercropping is cropping two or more different crops simultaneously

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in a portion of each crop life cycle. The second crop is planted after the first crop has reached the reproductive growth stage before reaching the harvest time (Palaniappan, 1985). The relay intercropping pattern of hot pepper and corn would be improved with the addition of biological agents to stimulate plant growth.

The relay intercropping of hot pepper and corn was chosen because, apart from being a food crop, corn has a different habitus from hot pepper, so it acts as a barrier from vector viruses and a place for predatory insects that attack hot pepper crops. Approximately 25 aphids were caught at each site in monoculture plot, while only 12 aphids were caught at each site in the intercropping plot of hot pepper with corn (Mitiku et al., 2014). Corn (C4) is a plant that is resistant to high light intensity, while hot pepper (C3) is a plant that requires a lower light intensity compared to C4 plants. According to Asharp and Sivachandiran (2018), partial shade significantly increased average plant height, number of leaves, fruit length, single fruit weight and total yield ( $\text{ton}\cdot\text{ha}^{-1}$ ) of hot pepper when compared to full sun. Relay intercropping is suitable to be applied in tropical areas with narrow fields, such as those commonly found in Indonesia, to maximize production and minimize the risk of crop failure caused by pests (Setiawan, 2009).

The commonly used biological agents includes Arbuscular Mycorrhizal Fungi, *Trichoderma* spp. and *Bacillus* spp. The benefits of Arbuscular Mycorrhizal Fungi application are the improvement in growth rate during seedling, crop uniformity, flowering, and yield. In addition, Arbuscular mycorrhizal fungi are able to increase crop resistance against biotic and abiotic stress through significant changes in hormone balance, as well as primary and secondary metabolism (Jung et al., 2012; Pereira et al., 2016). The application of *Trichoderma* promotes growth from the beginning of the crop nursery and shows the best response in biocontrol against disease (Herrera-Parra et al., 2017). The application of *Bacillus* spp. on pepper crops increases growth characteristics such as number of fruit and fruit weight (Datta et al., 2011), while, in corn, it increases crop biomass, relative water content, leaf water potential, and aggregate stability and reduces leaf water loss. Meanwhile, the application of *Bacillus velezensis* B-27 on snack fruit increases fruit length and fruit diameter (Ilmiah et al., 2021).

In this research, two models of the relay intercropping pattern of hot pepper and corn were used,

namely two rows of hot pepper in one bed and three rows of hot pepper in one bed. The advantage of the three rows hot pepper cropping model in one bed was the greater population. Thus, it was presumed that it could produce higher productivity of hot pepper. The combination of relay intercropping patterns with the application of biological agents was expected to be more optimal for the growth and yield of hot pepper, which then could be applied as an effort to achieve food security and optimized agricultural land use. This research aimed to determine the effect of relay intercropping and application of biological agents on the growth and yield of hot pepper.

## MATERIALS AND METHODS

### Research design

The research was conducted on agricultural land owned by farmers in Ketunggeng Village, Dukun District, Magelang Regency, Central Java Province, Indonesia, located at  $7^{\circ}34'17.4''\text{S}$  and  $110^{\circ}18'33.1''\text{E}$ , and at an altitude of  $\pm 578$  m above sea level (ASL) from May 2019 to January 2020. The morphophysiological analysis was carried out at the Laboratory of Crop Management and Production, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta. The materials used were local hot pepper cultivar Tiyung and hybrid sweet corn cultivar Talenta F1.

The research was arranged in a Factorial Randomized Complete Block Design (Factorial RCBD), with two factors, one control, and three replications in the same area as a block. The first factor was the relay intercropping pattern (two rows and three rows of hot pepper), and the second factor was the application of biological agents (with and without biological agents). The biological agents used were *Trichoderma asperellum*, *Bacillus velezensis* B-27, and Arbuscular Mycorrhizal Fungi (AMF). Four treatment combinations were obtained, including the three hot pepper rows cropping model with biological agents application, two hot pepper rows cropping model with biological agents application, three hot pepper rows cropping model without biological agents, and two hot pepper rows cropping model without biological agents. The control was a monoculture hot pepper without the application of biological agents.

The two hot pepper rows cropping model consisted of, two rows of hot pepper plants with a spacing of  $70\text{ cm} \times 60\text{ cm}$  and two rows of corn plants with a

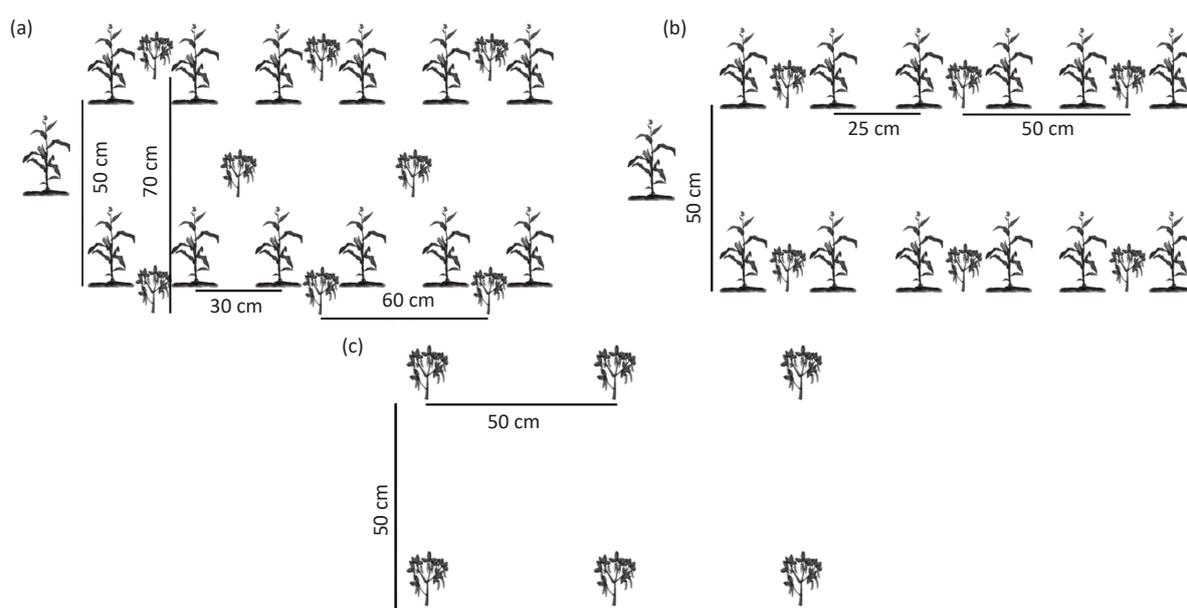
spacing of 50 cm × 30 cm. Meanwhile, the three hot pepper rows cropping model consisted of three rows of hot pepper plants and two rows of corn plants, with a spacing of 50 cm × 50 cm and 50 cm × 25 cm, respectively. In the three hot pepper rows model, there were three rows of hot pepper plants, where one row was in the middle and cropped cross-wise from two hot pepper rows which was on the edge and two corn rows were cropped parallel or equal to the three hot pepper rows. In model two hot pepper rows in one bed, there were two hot pepper rows and two corn rows cropped parallel to the hot pepper. In the monoculture cropping pattern of hot pepper in one bed, there were two rows of crops with a spacing 50 cm × 50 cm (schematic of the cropping model could be seen in Figure 1). Production plots were 3 m<sup>2</sup> (1 m × 3 m) for relay intercropping and monoculture. The hot pepper population per production plots were 14 plants in the three rows model, 12 plants in the two rows model, and 12 plants in monoculture. The population of corn per production plots in the three rows model were 20 plants, the two rows model were 24 plants and the monoculture were 24 plants.

### Research procedure

The research was carried out in Ketunggeng Village, Dukun District, Magelang Regency, Central Java Province, Indonesia. The research sites were planted with lowland paddy in the previous season.

The application of biological agents was carried out in the relay intercropping models. *Trichoderma asperellum* was given during tillage at the beginning of cultivation. Arbuscular Mycorrhizal Fungi was given twice, namely during seedling and transplanting. *Bacillus velezensis* B-27 was given when soaking the seeds and every other week after transplanting. During tillage, the soil was given manure, NPK (15 % N: 15 % P<sub>2</sub>O<sub>5</sub>: 15 % K<sub>2</sub>O, 9 % S and 2000 ppm Zn), and dolomite at a dose of 10 ton.ha<sup>-1</sup>, 400 kg.ha<sup>-1</sup>, and 1 ton.ha<sup>-1</sup>, respectively. *Trichoderma asperellum* with a fungus density of 2.2 × 10<sup>6</sup> cell.mL<sup>-1</sup> was applied by sprinkling them on the beds at 60 kg.ha<sup>-1</sup>. Each treated bed was covered with black silver plastic mulch and given a hole with a diameter of 10 cm for the cropping hole. Before the nursery, both hot pepper and corn seeds were soaked in *Bacillus velezensis* B-27 with a bacterial density of 10<sup>8</sup> cfu.mL<sup>-1</sup> at a dose of 10 mL.L<sup>-1</sup> of water, for 1 hour and then drained. The hot pepper and corn seeds were then sown for 30 days (4 weeks) and 14 days (2 weeks), respectively, and 1 g per hole of Arbuscular Mycorrhizal Fungi with a fungi density of 16 spores per gram of zeolite was applied.

When transplanting in the relay intercropping models, the corn seeds were transplanted first. After 2 weeks, hot pepper seeds were relay intercropped with corn. When transplanting, Arbuscular Mycorrhizal Fungi was applied at a dose of 3 g per hole. Application of *Bacillus velezensis* B-27 with a



**Figure 1.** Schematic of the intercropping models: (a) three hot pepper rows : two corn rows relay intercropping; (b) two hot pepper rows : two corn rows relay intercropping; and (c) hot pepper monoculture.

bacterial density of  $10^8$  cfu.mL<sup>-1</sup> of water was given at a dose of 10 mL.L<sup>-1</sup> of water ( $\pm 200$ –220 mL per crop) once every other week.

The follow-up fertilization for hot pepper plants was applied using NPK 15:15:15 and SP-36. NPK was applied at a dose of 400 kg.ha<sup>-1</sup>, which was divided into four times applications, namely 2, 5, 8, and 16 weeks after transplanting (WATP) at a dose of 40 kg.ha<sup>-1</sup>, 60 kg.ha<sup>-1</sup>, 150 kg.ha<sup>-1</sup>, and 150 kg.ha<sup>-1</sup>, respectively. SP36 was applied at a dose of 200 kg.ha<sup>-1</sup>, which was divided into four times applications, namely 2, 5, 8, and 16 WATP at a dose of 20 kg.ha<sup>-1</sup>, 30 kg.ha<sup>-1</sup>, 75 kg.ha<sup>-1</sup>, and 75 kg.ha<sup>-1</sup>, respectively. Meanwhile, the follow-up fertilization for corn plants was applied using NPK 15:15:15 at a dose of 300 kg.ha<sup>-1</sup>, which was divided into two times applications, namely a week and three weeks after transplanting at a dose of 150 kg.ha<sup>-1</sup> each (Widodo et al., 2016; Suherman et al., 2018).

Sweet corn plants were harvested  $\pm 8$  WATP. Corn stems were not removed, and corn leaves were trimmed  $\pm 1$  week after harvested. Hot pepper plants were harvested  $>12$ –28 WATP. In the relay intercropping treatment, hot pepper plants got a shading effect from corn plants for  $\pm 30$  days.

Observation of the light interception was carried out at 2–4 WATP when the hot pepper was relay intercropped with corn and at 6 WATP after corn harvested. The percentage of light interception (LI) was calculated by the following equation:

$$LI (\%) = \left[ 1 - \left( \frac{Q_b}{Q_m} \right) \right] \times 100\%$$

LI is the light interception,  $Q_m$  is the radiation range above the hot pepper canopy, and  $Q_b$  is the radiation reaching the ground level (Portes and de Melo, 2014). The hot pepper plant height was measured at 2–16 WATP at every other week (cm). A leaf sample for observation of relative water content at the age of 8 WATP was made up of ten leaves from the same plant of hot pepper. Relative water content (RWC) was calculated by the following equation:

$$RWC (\%) = \left[ \frac{(FM - DM)}{(TM - DM)} \right] \times 100\%$$

FM is fresh mass, DM is dry mass, and TM is turgid mass of the tissues (Barrs and Weatherley, 1962). Measurements of leaf greenness was performed using a SPAD 502 Plus Chlorophyll Meter (Konica Minolta, 2009). Measurements of stomatal density of adaxial leaf surface (unit per mm<sup>2</sup>) and size of stomatal

opening of abaxial leaf surface ( $\mu\text{m}$ ) was carried out using a microscope with 100 $\times$  magnification lens and 400 $\times$  magnification lens, respectively, at the age of 8 WATP. The number of branches, number of leaves, total root length (cm), total dry weight of plants (g), and root-shoot ratio were observed at the age of 8 WATP. Consumption index, a ratio between the economic fresh weight ( $W_e$ ) and the total fresh weight of the plant ( $W$ ), was observed at the age of 8 WATP. Net assimilation rate (NAR, mg.cm<sup>-2</sup>.weeks<sup>-1</sup>) was calculated at the age of 8 WATP by the following equation:

$$NAR = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\ln La_2 - \ln La_1}{La_2 - La_1}$$

$W$  is total fresh weight,  $T$  is time, and  $La$  is leaf area (Williams, 1946). Observations of the number of fruits per plant, weight of fruits per plant (g per plant) and productivity (ton.ha<sup>-1</sup>) were carried out from beginning to the end of harvest period.

#### Data analysis

Data were analyzed using Analysis of Variance (ANOVA), followed by the Post Hoc Duncan's Multiple Range Test (DMRT) the  $\alpha$  level of 5 %. The statistical analysis was performed using SAS 9.4 program.

## RESULTS AND DISCUSSION

### Light interception of hot pepper

Based on the research conducted, the monoculture of hot pepper produced the highest percentage of light interception at 2 weeks after transplanting with the highest value reaching 41.5 %, but not significantly different compared to that in the relay intercropping pattern (Figure 2). Light interception is one of the parameters characterizing resource capture, including in double cropping, such as relay intercropping. A high percentage of light interception is affected by high light intensity. According to Kurniaty et al. (2010), the higher light intensity affects the activity of leaf stomata cells in reducing transpiration, thus potentially inhibiting crop growth, while too low light intensity produces photosynthates that was not optimal, thus inhibiting crop growth. In the relay intercropping treatment, hot pepper crops got a shading effect from corn crops for  $\pm 30$  days (during 4 WATP) so that the percentage of light interception at the beginning of the hot pepper vegetative

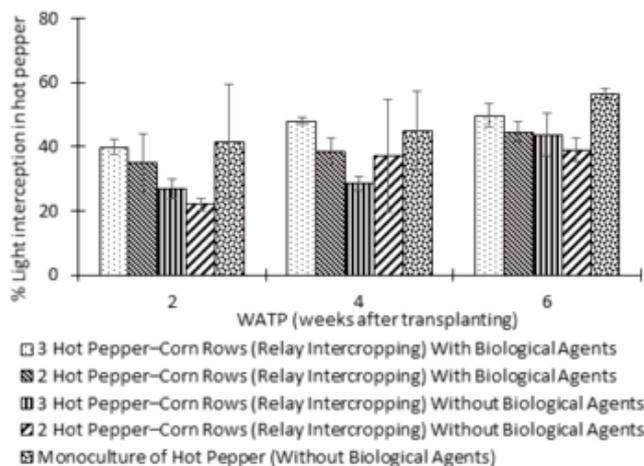


Figure 2. Percentage of light interception in hot pepper as affected by intercropping models and biological agents treatments.

growth for all treatments in relay intercropping was less than 47.9 % (at 4 WATP).

The light interception in the relay intercropping of hot pepper-corn given biological agents in both two and three rows models was greater than that without biological agents, namely 47.9 % for three rows model and 38.5 % for two rows model at 4 WATP. This is because biological agents could increase the canopy of hot pepper plants. According to Geetha et al. (2019), the quantity of radiation captured by the crop canopy is influenced by a number of factors such as leaf angle, leaf surface properties that affect light reflection, leaf thickness and chlorophyll, which affect light transmission, leaf size and shape, sun height, and direct distribution of scattered sun light. The relay intercropping of hot pepper-corn had a more complex canopy model and caused a shading effect for up to ±30 days (4 WATP). Thus, after the corn plants were harvested, the application of biological agents could trigger the growth of hot pepper since the percentage of light interception increased starting from 6 WATP until the end of vegetative phase.

**Relative water content, leaf greenness, stomatal density of abaxial leaf surface and size of stomatal opening of adaxial leaf surface of hot pepper**

There was no interaction between the intercropping model and biological agents on the relative water content and leaf greenness of hot pepper. However, there was no significant effect of the relay intercropping of hot pepper-corn on the relative water content and leaf greenness of hot pepper to. Conversely, the application of biological agents did not significantly affect the relative water content and leaf greenness

of hot pepper compared to those without biological agents (Table 1). According to Lugojan and Ciulca (2011), relative water content is calculated to determine the status of water in crop tissue, which reflects the balance between water availability to leaf tissue and the rate of transpiration. In general, the relative water content of hot pepper crops ranged from 91.17 % to 94.25 %, which means that the crop only experiences mild stress. According to Barr and Weatherley (1962), the normal value of relative water content is ±98 %. A value of 80 % to 98 % shows mild stress, while the value of 60 % to 70 % indicates that the crop experiences moderate stress.

An approach to determine the amount of leaf chlorophyll is to measure leaf greenness using the SPAD 502 Plus Chlorophyll Meter tool. This tool records the leaf greenness and the relative number of chlorophyll molecules present in the leaves at a value based on the amount of light transmitted by the leaves (Konica Minolta, 2009). In the relay intercropping model, the percentage of light interception obtained was well utilized by the crops. Thus, the low percentage of light interception did not affect the leaf greenness (Table 1). The percentage of light interception in the relay intercropping pattern, which was lower than that in the monoculture, resulted in the same relative water content and leaf greenness of hot pepper as in the monoculture pattern.

The results showed that there was no interaction between the intercropping models and biological agents on the stomatal density of the adaxial leaf surface and the size of stomatal opening of the abaxial leaf surface of hot pepper (Table 1). The stomatal density of the adaxial leaf surface in relay

**Table 1.** Relative water content, leaf greenness, stomatal density of abaxial leaf surface and size of stomatal opening of adaxial leaf surface of hot pepper with crop models and biological agents treatments at 8 WATP

Treatments	Relative water content (%)	Leaf greenness (unit)	Stomatal density of adaxial leaf surface (unit per mm <sup>2</sup> )	Size of stomatal opening of abaxial leaf surface (µm)
Monoculture of hot pepper	94.25 a	50.29 a	22.67 b	14.25 a
Relay intercropping of hot pepper-corn	92.07 a	47.87 a	35.50 a	15.07 a
Intercropping models (Relay intercropping)				
2 hot pepper-corn rows	91.17 p	46.57 p	31.50 p	14.15 p
3 hot pepper-corn rows	92.96 p	49.16 p	39.50 q	15.99 q
Biological agents				
With biological agents	92.26 r	47.37 r	43.72 r	17.08 r
Without biological agents	91.87 r	48.37 r	27.28 s	13.07 s
Interaction	(-)	(-)	(-)	(-)
Coefficient of variations (%)	5.13	10.83	13.74	6.74

Remarks: Means followed by the different letters in the same column are significantly different based on DMRT, at α= 5 %, (-) sign shows no interaction between the intercropping models and biological agents, WATP= Weeks After Transplanting.

intercropping of hot pepper-corn was significantly higher compared to that in the monoculture of hot pepper. Crops adapt to lower light intensity by producing wider, thinner leaves with thinner epidermal layers, less palisade tissue, larger space between cells, and more stomatal numbers (Libria et al., 2004). There was significant difference in the stomatal density of adaxial leaf surface as affected by the relay intercropping of hot pepper-corn with three rows model and the application of biological agents (Table 1). The application of biological agents significantly increased the stomatal density of the adaxial leaf surface of hot pepper. This result was in accordance with the research of Cappellari et al. (2015), reporting that the treatment of biological agents was useful to increase the stomatal density of mint crops.

Sunlight affects the opening and closing of stomatal, thereby affecting the size of the stomatal openings. The average of the width and length of the stomatal opening was used to determine the size of the stomatal opening. The relay intercropping of three rows model and the application of biological agents significantly increased the size of stomatal opening of abaxial leaf surface. The relay intercropping of three rows model had a significantly higher size of stomatal opening of abaxial leaf surface (15.99 µm) than that in the relay intercropping of two rows model (14.15 µm). Furthermore, Table 1 also showed that the application of biological agents had a significantly higher size of stomatal opening of abaxial leaf surface (17.08 µm) than that without application of

biological agents (13.07 µm).

Non-homogeneous shade derived from crops (corn) could reduce stress, thereby increasing or maintaining the size of the stomatal opening. The application of biological agents was useful in influencing the size of the abaxial surface leaf stomatal opening because these microbes acted as nutrient providers, growth regulating metabolites, and bioactivators (Saraswati and Sumarno, 2008). According to Azoulay-Shemer (2015), stomatal opening could be regulated by physiological and environmental factors, in particular CO<sub>2</sub>, abscisic acid (ABA), humidity, dryness, ozone, and pathogens. Controlled environmental conditions from pathogens would support optimal stomatal opening. The relay intercropping did not inhibit physiological processes. The wider stomatal opening could support a stronger rate of CO<sub>2</sub> absorption for the photosynthesis process, thereby potentially increasing plant growth and yield of hot pepper.

**Plant height of hot pepper**

Plant height is measurement that is frequently used to determinecrop growth. The sigmoid curve of hot pepper plant height growth from 2–16 WATP (weeks after transplanting) is presented in Figure 3. In the figure, the the relay intercropping of two and three rows models with the application of biological agents showed higher growth compared to the relay intercropping models without the application of biological agents and monoculture of hot pepper until the end of the vegetative phase. Plant height

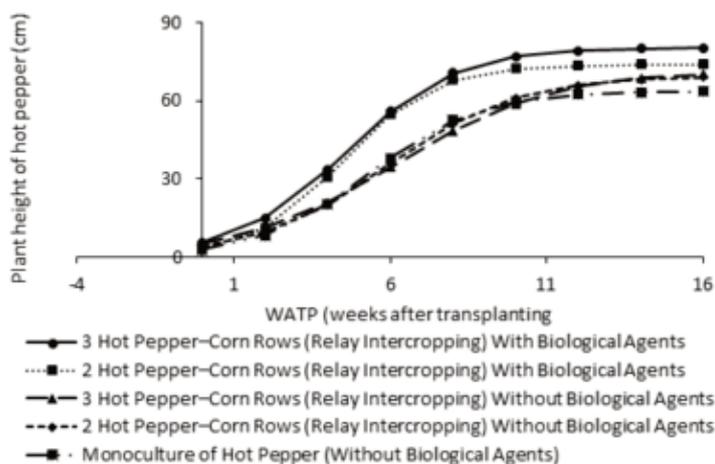


Figure 3. The sigmoid curve of hot pepper plant height as affected intercropping models and biological agents treatments.

Table 2. Number of branches, number of leaves and total root length of hot pepper as affected by intercropping models and biological agents treatments at 8 WATP

Treatments	Number of branches	Number of leaves	Total root length (cm)
Monoculture of hot pepper	6.17 a	218.33 a	2074.48 a
Relay intercropping of hot pepper-corn	3.29 b	81.67 b	1626.97 a
Intercropping models (Relay intercropping)			
2 hot pepper-corn rows	3.50 p	81.08 p	1892.90 p
3 hot pepper-corn rows	3.08 p	82.25 p	1361.10 p
Biological agents			
With biological agents	3.08 r	86.17 r	1722.47 r
Without biological agents	3.50 r	77.17 s	1531.47 r
Interaction	(-)	(-)	(-)
Coefficient of variations (%)	20.51	20.05	29.99

Remarks: Means followed by the different letters in the same column are significantly different based on DMRT, at  $\alpha = 5\%$ , (-) sign shows no interaction between the intercropping models and biological agents, WATP= Weeks After Transplanting.

growth began to differ at 10 WATP, in which the relay intercropping of three rows model with the application of biological agents resulted in the higher growth compared to other treatments. The application of biological agents had an effect on the plant height when viewed from the growth curve at 4–12 WATP. According to the results of research by Samaniego et al. (2016), hot pepper crops treated with biological agents had more nutrient content, so they could be absorbed by crops for metabolic processes and plant height growth of hot pepper. The monoculture of hot pepper showed the lowest plant height compared to all the treatments tested. Although the percentage of light interception was the highest, the resulting plant height growth showed the opposite result. According to Geetha et al. (2019),

the use of light energy for growth in multiple cropping systems was more effective than in monoculture.

### Number of branches, number of leaves, and total root length of hot pepper

The number of productive branches reflects the hot pepper fruit sets number because the fruit organ functions as the highest sink that receives assimilation from the photosynthetic process (Ganefianti et al., 2019). The results showed that there was no interaction between the intercropping models and biological agents on the number of branches and the number of leaves of hot pepper. In relay intercropping of hot pepper-corn, the number of branches and the number of leaves were significantly smaller than in the monoculture of hot pepper.

The relay intercropping of two rows model did not give significantly different effect compared to the three rows model (Table 2). The number of branches affected the number of leaves produced by hot pepper. The fewer the number of branches, the fewer the number of leaves. The number of leaves is related to the assimilates from photosynthesis, in which the more the leaves, the more assimilates the crop produces for the crop development, resulting in the greater dry matter production. The application of biological agents did not affect the number of branches produced by hot pepper, but it affected the number of leaves. According to Madusari et al. (2018), the application of biological agents had a significant effect on increasing plant growth, such as the number of leaves.

Root length is one of the crop growth parameters obtained by measuring the total root length of the crop. The results showed that there was no interaction between the cropping models and biological agents on the total root length of hot pepper. The relay intercropping of hot pepper-corn did not give significantly different effect compared to the monoculture of hot pepper. The total root length produced by the relay intercropping of two and three rows model was not significantly different (Table 2). This showed that the relay intercropping model of hot pepper with corn did not inhibit the growth of hot pepper crop roots. Microorganism was useful as a biological agent that could control crop pathogens, as well as increase the growth and productivity with direct or indirect

effects, such as providing a source of N for crops through N fixation, using biological control for pathogens in the soil, and producing phytohormones (Piromyou et al., 2011). The application of biological agents did not significantly give different effect on the total root length. According to Yanti et al. (2017), the interaction between a consortium of biological agents with crops could become unstable so that their role was not significant in the growth of the hot pepper roots.

**Total dry weight, root-shoot ratio, net assimilation rate, and consumption index of hot pepper**

There was no interaction between the intercropping models and biological agents on the total dry weight of hot pepper. The total dry weight of hot pepper in the relay intercropping model of two rows was not significantly different from that in the three rows model (Table 3). These results were influenced by the number of branches and the number of leaves. This was also due to the lower percentage of light interception, resulting in a significantly smaller total crop dry weight as well. The dry weight of plants was the result of CO<sub>2</sub> absorption during the photosynthesis and CO<sub>2</sub> release during the respiration process. The lower the respiration carried out by the plants, the higher the net photosynthesis produced. The high photosynthetic rate results in the increase in the plant dry weight (Ahmadi et al., 2014).

There was no interaction between the intercropping models and biological agents on the root-shoot ratio

**Table 3.** Total dry weight, root-shoot ratio, net assimilation rate and consumption index of hot pepper as affected by intercropping models and biological agents treatments at 8 WATP

Treatments	Total dry weight (g)	Root-shoot ratio	Net assimilation rate (mg.cm <sup>-2</sup> .weeks <sup>-1</sup> )	Consumption index
Monoculture of hot pepper	56.02 a	0.05 a	10 a	1.07 a
Relay intercropping of hot pepper-corn	22.44 b	0.09 a	10 a	1.01 a
Intercropping models (Relay intercropping)				
2 hot pepper-corn rows	23.87 p	0.10 p	10 p	0.90 p
3 hot pepper-corn rows	21.02 p	0.08 p	10 p	1.11 p
Biological agents				
With biological agents	20.54 r	0.10 r	10 r	1.18 r
Without biological agents	24.35 r	0.08 r	10 r	0.83 r
Interaction	(-)	(-)	(-)	(-)
Coefficient of variations (%)	24.37	16.40	13.10	17.56

Remarks: Means followed by the different letters in the same column are significantly different based on DMRT, at α= 5 %, (-) sign shows no interaction between the intercropping models and biological agents, WATP= Weeks After Transplanting.

**Table 4.** Number of fruits per plant, weight of fruits per plant and productivity of hot pepper as affected by intercropping models and biological agents treatments.

Treatments	Number of fruits per plant	Weight of fruits (g per plant)	Productivity of hot pepper (ton. ha <sup>-1</sup> )
Monoculture of hot pepper	193.68 a	466.42 a	11.16 a
Relay intercropping of hot pepper-corn	183.59 a	406.36 a	12.93 a
Intercropping models (Relay intercropping)			
2 hot pepper-corn rows	183.13 p	385.02 p	12.02 p
3 hot pepper-corn rows	184.06 p	427.70 p	13.85 p
Biological agents			
With biological agents	228.96 r	486.97 r	16.84 r
Without biological agents	138.23 r	325.76 s	9.03 s
Interaction	(-)	(-)	(-)
Coefficient of variations (%)	14.79	10.36	14.16

Remarks: Means followed by the different letters in the same column are significantly different based on DMRT, at  $\alpha = 5\%$ , (-) sign shows no interaction between the intercropping models and biological agents.

of hot pepper. The root-shoot ratio of hot pepper in the relay intercropping of two rows model was not significantly different from that in the three rows model. The application of biological agents also did not give significant effect on the root-shoot ratio of hot pepper (Table 3). The root-shoot ratio was lower than one because the shoot biomass was greater than that of plant roots. Although it was not significantly different, the root-shoot ratio in the monoculture was smaller than that in the relay intercropping, and the root-shoot ratio without biological agents was smaller than with the application of biological agents. It showed that the shoot or canopy of hot pepper in monoculture pattern was greater than the shoot of hot pepper in relay intercropping pattern. However, the root of hot pepper in monoculture pattern was as large as the root of hot pepper in relay intercropping pattern.

There was no interaction and there was no significant difference between the intercropping models and biological agents on the net assimilation rate and the consumption index of hot pepper (Table 3). The net assimilation rate, which was not significantly different, proved that the relay intercropping pattern did not inhibit plant growth because the assimilation produced by plants per unit of leaf area per unit time was as high as with hot pepper monoculture. Consumption index higher than one indicates that the economic fresh weight of hot pepper per plant was greater than the total fresh weight of hot pepper. The same consumption index

in monoculture and relay intercropping indicated that the hot pepper in the relay intercropping pattern was more efficient in using assimilates translocated from the photosynthesis for the formation of fruits. According to Maure et al. (2019), net assimilation rate in monoculture pattern was not significantly different from that in the intercropping pattern, and the yield per plant in the intercropping pattern increased by 12.66 % to 19.52 % compared to that in monoculture.

#### Number of fruit per plant, weight of fruit per plant and productivity of hot pepper

There was no interaction between the cropping models and biological agents on the number of fruits per plant, weight of fruits per plant, and productivity of hot pepper (Table 4). The total dry weight of hot pepper crops in relay intercropping model was smaller than that in the monoculture of hot pepper. However, the number of fruits per plant and weight of fruits per plant produced in the relay intercropping model could be equivalent to those in the monoculture of hot pepper. The results showed that hot pepper plants in relay intercropping pattern were more efficient in utilizing assimilates from the photosynthesis for the formation of generative organs, including fruits. The stem diameter, the number of branches and canopy width in the monoculture of hot pepper were significantly higher than in the intercropping (Ganefianti et al., 2019). According to Ahmed et al. (2016), the intercropping of hot pepper-corn could give a yield of number of fruits per plant and weight

of fruits per plant which was equivalent to the yield in the monoculture of hot pepper. This showed that the relay intercropping model did not inhibit growth and production of hot pepper per plant. This result was also related to the role of corn in the relay intercropping patterns as a barrier crop that could reduce aphids infestation, which could decrease hot pepper fruit production (Mitiku et al., 2014).

Table 4 also shows that the relay intercropping of two and three rows model produced hot pepper productivity that was statistically comparable to the monoculture of hot pepper. Bigger plant population in the three rows model was able to increase productivity, but not significantly different compared to the productivity of hot pepper in the two rows model. Meanwhile, the relay intercropping resulted in the lower growth rate and total dry weight compared to those in the monoculture of hot pepper. According to Pratiwi (2016), the bigger crop population results in the fewer tillers and lower growth of vegetative organs. In a smaller plant population, the growth of vegetative organs was higher, but the yield of land area could be lower. In this research, the productivity in relay intercropping was 12.93 ton.ha<sup>-1</sup>, which was 15.8 % higher than in monoculture (11.16 ton.ha<sup>-1</sup>).

The application of biological agents significantly increased the weight of fruits per plant and productivity of hot pepper compared without biological agents. The plant productivity as affected by the application of biological agents (*Trichoderma asperellum*, *Bacillus velezensis* B-27 and Arbuscular Mycorrhizal Fungi) was 16.84 ton.ha<sup>-1</sup>, which was 86.50 % higher than without biological agents application (9.26 ton.ha<sup>-1</sup>). According to Syafruddin (2017), giving biological agents to hot pepper crops could increase growth and yield because it could provide available P to be utilized by crops. Although there was no interaction between the two treatments tested, this research could provide information that relay intercropping of hot pepper-corn could produce the same hot pepper productivity as in the monoculture. The relay intercropping of two and three rows model and the application of biological agents could be applied as an effort to increase food security and optimize agricultural land use.

## CONCLUSIONS

Relay intercropping with the application of biological agents increased the light interception. Relay

intercropping of hot pepper produced lower number of branches, lower number of leaves, and lower total dry weight. However, the plants in the intercropping models were more efficient in the use of assimilates for the development of generative organs based on net assimilation rate and consumption index, resulting in the large number of fruit per plant and higher weight of fruit per plant produced, which were statically comparable to those in the monoculture of hot pepper. The application of biological agents significantly increased the stomatal density, the size of stomatal opening, the number of leaves, weight of fruits per plant, and productivity of hot pepper.

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