Effort to increase off-season production and fruit quality of Siam orange (*Citrus nobilis var. microcarva* L.) through application of mycorrhizal inoculants and auxin

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Abstract. The study was conducted in Belancan Village, Kintamani, Bangli, Bali, from February to October 2018 with the aim to study the effect of application of mycorrhizal inoculants and auxin to increase off-season production and fruit quality of Siam orange. This experiment used a randomized block design with 2 factors. The first factor mycorrhizal inoculant dosages (0, 50, and 100 g/tree), while the second factor auxin concentrations (0, 50, 100 and 150 ppm/tree), and repeated 3 times. The results showed that the interaction of mycorrhizal inoculant dose and auxin concentration did not significantly effect to all of observed variables. The dose of mycorrhizal inoculant 100 g/tree increased off-season fruit production and quality of fruits, which was reflected by increase number of fruits per tree (142.17 fruits), weight per fruit (96.00 g), weight of fruit per tree (13.53 kg), and total dissolved solids (14.10 %Brix), or an increase of 44.21%; 11.89%; 57.87% and 21.03% compared to without mycorrhizal inoculant (108.58 pieces; 88.80 g; 10.57 kg and 11.65 %brix). Concentration of auxin 100 ppm/tree increased off-season production and quality of fruits, reflected by the higher number of fruits/trees (155.22 fruits), fruit weight per tree (15.03 kg), weight per fruit (96.50 g) and total dissolved solids (14.43% brix), compared to without auxin i.e. 112.11 pieces; 10.44 kg; 84.66 g; and 11.15% brix. The significant result of this research was mycorrhizal inoculant dose of 100 g/tree and auxin in the form of IBA 100 ppm/tree proven fruit drop, increased off-season fruit production and quality of fruits of Siam orange.

1. Introduction
Siam orange (*Citrus nobilis var. Microcarpa* Hassk) is one of the most widely cultivated oranges in Indonesia [1]. Citrus farming provides great benefits because oranges are consumed by various consumer groups, not only served as a table fruit that is enjoyed in a fresh state, processed as a beverage in packaging, as a source of vitamin C needed by the body, but also be processed into other processed ingredients. In Bali, fruit oranges are also used as offerings “gebogans” in Hindu religious rituals [2]. Although the price of oranges on market is unstable, business opportunities for cultivation of Siam orange is still favorite for some people, because they can be cultivated in the lowlands and highlands. If the cultivation is done well, Siam oranges will have high production with high quality and have a high price. The market demand of Siam oranges fruits is high and its promising price causes the interest of the community to cultivate oranges very high [3].
Constraints faced by the on cultivating of Siam orange is the production seasonally and quality of the fruit low so affecting price fluctuations, thus in terms of agribusiness not profitable. Potential yield of Siam orange can reach 25 tons/ha [4], but according to Januwiata et al., [5] at the production center in Payangan-Gianyar Regency, yiled of Siam orange is only 2.9 tons/ha or 40-70 kg/trees. This happens because maintenance is not adequate; especially fertilization that is not in accordance with the recommendations, both regarding the type of fertilizer and the time and method, and no action can be taken to stimulate flower induction such as giving growth regulators and maintenance properly.

One of the most prospective efforts to overcome the problems faced by Siam citrus farmers is to use mycorrhizal biofertilizer [6]. Mycorrhiza is a fungus that forms a mutualistic symbiotic relationship with plant roots, increase the absorption of nutrients, especially P, also increase drought resistance, attacks root pathogens and produce growth hormones such as cytokines, so that it can help plants in unfavorable soil conditions [7,8]. It was further stated that in addition to increasing mycorrhizal P-element adsorption, plants with micorhiza could also extract N, K, S, Ca, Mg, Zn and Si even in small amounts. Researchers [9-11] states that mycorrhiza that infects plant roots will produce intensive hyphae branching in soils that are spread very widely beyond the absorption area of root hairs so that plants are able to increase their capacity to absorb water and nutrients. Another advantage of giving mycorrhiza is that once a plant is infected by mycorrhiza, the benefits for plants will be obtained during his life. Mycorrhiza can be left in the soil even though the parent plant is gone [12]. The association between plant roots and mycorrhizal fungi provides very good benefits for the soil and host plant which is where the fungus grows and reproduces [13-15]. Utilization of mycorrhizal fungi, which are fungi that are associated with roots and function to help plants in marginal/less fertile areas in absorbing nutrients and water and as a source of biological fertilizer/biofertilizer, are expected to be able to help and facilitate citrus farmers to produce off-season fruits so that the obstacles faced can be overcome. The research results of Juliadewi et al., [16] that zalacca plants fertilized with endomycoriza biofertilizer at a dose of 75 g/tree increased production and could produce off-season fruit. It was stated that this was related to increase of percentage of fruit-set (percentage of flowers that develop into fruit) in plants that received mycorrhizal treatment compared to control, which was 77.22% in the “Gadu” season and “85.98%” in the “Sela II” season, and it was positively correlated with longer and wider root infected with mycorrhizae so that its ability to absorb water and nutrients is greater. On Siam orange Astiari et al., [6] found that mycorrhizal inoculants dose of 150 g/tree decreased flower drop so increased the percentage of flowers develop to be fruits.

Another problem faced by farmers Siam orange is the decline of fruit production due to fall of flowers and young fruits from the tree. Efforts to overcome the occurrence can be done by sprouting several types of growth regulators or plant hormones to maximize plant productivity. Growth regulators can increase the number of flowers and fruits and accelerate the fruit growth and development so does not fall down. Groups of growth regulators such as auxin, among others, play a role in stimulating cell division, increased plasticity and elasticity of cell walls, regulate flowering and fruiting, and prevent flower and fruit drop [17]. Auxin functions to stimulate cell extension, flower and fruit formation, prolong the point of growth, and prevent leaf and fruit fall [18]. Indole Butiric Acid (IBA) is a synthetic hormone that belongs to the auxin group, besides being used to stimulate root formation, also has other benefits such as increasing sprouts, stimulate fruit development, prevent drop of flowers and fruit, encourage cambium and other activities [19]. The results of the research [20] in zalacca plants during off-season period indicated that the treatment of IBA with a concentration of 100 ppm can significantly increase the number of fruits and fruit weight per tree, respectively 43.67 fruits and 2011.70 g, or increase of 70.25% and 85.11% compared to without IBA which was 13.00 fruits and 299.36 g, respectively.

As described above, the application of mycorrhizal inoculants and auxin was chosen to proven fruit drop, and increased off-season fruit production and quality of fruits of Siam orange.
2. Material and methods
The study was conducted in Belancan Village, Kintamani District, Bangli Regency, Bali Province, from February to October 2018. This experiment used factorial randomized block design (RBD) consisting of 2 factors. The first factor was mycorrhizal inoculant dose, namely: \( M_0 \) (without mycorrhizal/control), \( M_1 \) (50 g/tree), and \( M_2 \) (100 g/tree), while the second factor was the concentrations of auxin hormone in the form of IBA (Indole Butyric acid) i.e. \( A_0 \) (0 ppm/tree), \( A_1 \) (50 ppm/tree), \( A_2 \) (100 ppm/tree and \( A_3 \) (150 ppm/tree). The combination treatment was repeated 3 times so that it took 36 sample plant trees. Variables observed were percentage of fruit-set per tree, leaf chlorophyll content, N and P leaf content, leaf relative water content, total sugar content of leaves, leaf reducing sugar, leaf sucrose, weight per fruit, number of fruits per tree, fruit diameter, weight of fruit per tree, and total dissolved solids. Data were analyzed statistically with analysis of variance according to the design used. If the F Test showed that the interaction has a significant effect, then to compare the values between treatments was used Duncan’s multiple range test, whereas if only the single factor has a significant effect, least significance different (LSD) was used.

3. Results and discussion
Based on statistical analysis, it was found that the interaction between mycorrhiza and concentration of auxin hormone had no significant effect on all off observed variables, whereas mycorrhizal inoculant and auxin hormone concentration had significant effect almost to all of observed variables. Mycorrhizal inoculant treatment of 100 g/tree gave the highest fruit weight and number of fruits per tree i.e. 13.53 kg and 142.17 fruits or increased by 57.87% and 44.22% compared to control (8.75 kg and 98.58 fruits per tree) (Table 2). Increasing fruit weight per tree in 100 g/tree mycorrhizal inoculant treatment supported by weight per fruit (96.00 g), number of fruits per tree (142.17 fruits) and fruit diameter (7.38 cm) or an increase of 11.89%; 44.22% and 10.81% compared to controls with values subsequently were 85.80 g; 98.58 pieces and 6.66 cm (Table 2). The higher number of fruits per tree in 100 g/tree mycorrhizal inoculants was caused by the higher of fruit-set percentage (93.89%) than control (85.75%), and higher percentage of fruit-set in 100 g/tree mycorrhizal inoculants caused higher number of fruits per tree and fruit weight compared to that of on 50 g/tree mycorrhizal inoculants and control.

Yield components per tree (number of fruits per tree, weight per fruit, and fruit diameter) and yields per tree (weight of fruit harvested per tree) on 100 g/tree mycorrhizal inoculants which was higher than these of on control, was related to higher relative water content of leaf on 100 g/tree mycorrhizal inoculants (89.09%) than that of on control (83.48%) (Table 1). In the opinion of [9] that, mycorrhizae that infect plant roots will produce intensive hyphae in the soil, spreading very wide (± 80 mm) outside the root hair absorption area, so plants can increase their capacity to absorb water and nutrients. This was reflected by the increase of relative water content of leaves. [7] stated that plants with mycorrhiza besides increasing the adsorption of the P nutrient, could also extract N, K, S, Ca, Mg, Zn and Si, although in smaller amounts. From the results of the study it could be proven that mycorrhizal inoculant with a dose of 100 g/tree increased leaf N nutrient uptake (1.81%) and leaf P nutrient (0.20%) compared to control i.e. 1.35% and 0.11%. Increased nutrient content of N and P leaves caused a better metabolic process, so increased leaf chlorophyll content which was 64.74 SPAD on 100 g/tree mycorrhizal inoculants compared to 54.53 SPAD on control (Table 1). Increased leaf chlorophyll content could increase the photosynthates produced, especially total leaf sugar, reducing sugar and leaf sucrose which were 27.40%; 8.42% and 19.06%, respectively and higher than these of on control with value subsequently 13.59%; 4.93% and 8.66%. The higher total sugar, reducing sugar and sucrose content in leaves reduced fruit drop and also improved fruit quality which was reflected by the higher total dissolved solids in fruit that was 14.10% brix on 100 g/tree mycorrhizal inoculants and only 11.65% brix on control (Table 2).
Table 1. Effect of mycorrhizal inoculants and auxin growth regulators on percentage of fruit-set, percentage of fruit drop, leaf chlorophyll content, leaf relative water content, N and P leaves content, and total sugar leaf content.

| Treatment | Percentag e of Fruit-Set (%) | Percentag e of fruit drop (%) | Chlorophyll leaves content (SPAD) | Leaf relative water content (%) | N leaves content (%) | P leaves content (%) | Total sugar leaf content (%) |
|-----------|-----------------------------|------------------------------|----------------------------------|---------------------------------|----------------------|----------------------|-------------------------------|
| Mycorrhizal (M) |                             |                              |                                  |                                 |                      |                      |                               |
| M₀        | 85.75 b                      | 17.39 a                      | 54.53 b                          | 83.48 b                         | 1.35 b               | 0.11 c               | 13.59 c                       |
| M₁        | 92.41 a                      | 10.08 b                      | 57.49 ab                         | 86.01 b                         | 1.65 a               | 0.16 b               | 21.75 b                       |
| M₂        | 93.89 a                      | 9.73 b                       | 64.74 a                          | 89.09 a                         | 1.81 a               | 0.20 a               | 27.40 a                       |
| BNT 5%    | 2.54                         | 4.03                         | 8.86                             | 2.79                            | 0.21                 | 0.03                 | 1.13                          |
| Auxin (A) |                             |                              |                                  |                                 |                      |                      |                               |
| A₀        | 86.17 c                      | 15.89 a                      | 49.44 b                          | 83.33 b                         | 1.28 b               | 0.09 c               | 17.76 c                       |
| A₁        | 90.22 b                      | 12.67 ab                     | 63.25 a                          | 85.03 b                         | 1.53 a               | 0.14 b               | 20.87 b                       |
| A₂        | 94.02 a                      | 8.84 b                       | 63.31 a                          | 88.65 a                         | 1.69 a               | 0.18 a               | 23.65 a                       |
| A₃        | 92.34 ab                     | 12.24 b                      | 59.67 a                          | 87.92 a                         | 1.68 a               | 0.18 a               | 22.65 a                       |
| BNT 5%    | 2.20                         | 3.49                         | 7.67                             | 2.66                            | 0.21                 | 0.03                 | 1.13                          |

Note: The average value followed by the same letter in the treatment and column same, it means that it is not significantly different at the BNT test level of 5%.

Table 2. Effects of mycorrhizal inoculants and auxin hormone concentrations on leaf reducing sugar content, leaf sucrose content, number of fruit per tree, diameter per fruit, weight per fruit, weight of fruit per tree and total dissolved solids.

| Treatment | Reduction sugar of leaves content (%) | Sucrose content of leaves (%) | Number of fruit per tree (fruits) | Diameter of fruit (cm) | Weight per fruit (g) | Fruit weight per tree (kg) | Total dissolved solid (%Brix) |
|-----------|---------------------------------------|------------------------------|----------------------------------|------------------------|----------------------|-----------------------------|-------------------------------|
| Mycorrhizal (M) |                                      |                              |                                  |                        |                      |                             |                                |
| M₀        | 4.93 c                                | 8.66 c                       | 98.58 b                          | 6.66 b                 | 85.80 b              | 8.57 b                     | 11.65 b                       |
| M₁        | 6.63 b                                | 14.61 b                      | 137.75 a                         | 7.25 ab                | 93.87 a              | ab                         | 13.45 a                       |
| M₂        | 8.42 a                                | 19.06 a                      | 142.17 a                         | 7.38 a                 | 96.00 a              | 13.53 a                    | 14.10 a                       |
| BNT 5%    | 0.44                                  | 1.64                         | 8.81                             | 0.42                   | 8.81                 | 3.16                       | 0.70                          |
| Auxin (A) |                                      |                              |                                  |                        |                      |                             |                                |
| A₀        | 5.88 c                                | 12.15 c                      | 102.11 b                         | 6.39 b                 | 81.66 b              | 8.44 c                     | 11.15 c                       |
| A₁        | 6.88 b                                | 13.95 b                      | 125.56 b                         | 7.11 a                 | 94.42 a              | 11.87 b                    | 13.50 b                       |
| A₂        | 9.23 a                                | 14.42 a                      | 155.22 a                         | 7.54 a                 | 96.50 a              | 15.03 a                    | 14.43 a                       |
| A₃        | 9.20 a                                | 13.45 a                      | 121.78 a                         | 7.35 a                 | 94.98 a              | 11.71 b                    | 13.38 a                       |
| BNT 5%    | 0.44                                  | 1.64                         | 7.63                             | 0.32                   | 7.63                 | 2.74                       | 0.70                          |

Note: The average value followed by the same letter in the same treatment and column, means that it is not significantly different at the BNT test level of 5%.

Treatment of auxin concentration of 100 ppm/tree gave the highest percentage of fruit-set (Table 1) and so number of fruits per tree, diameter of fruit and weight per fruit (Table 2). Increasing number of fruits, fruit diameter and weight per fruit on 100 ppm/tree auxin concentration gave higher fruit weight per tree compared to 50 ppm/tree auxin and control. The yield and yield component per tree was higher at a concentration of 100 ppm/auxin tree due to an increase in leaf relative water content, leaf chlorophyll content, N and P leaves content than those of controls which lead to more optimal photosynthetic activity so that the resulting photosynthate was higher, represented by total sugar content, reducing
sugar, leaf sucrose and higher total dissolved solids (Table 1 and Table 2). Increasing the total dissolved solids in the fruit caused fruit taste sweeter so the quality of the fruit be improved. [18] stated that Indole Butiric Acid (IBA) is one of the synthetic hormones included in the auxin group, besides being used to stimulate root formation, it also has other benefits such as stimulating fruit development, preventing the fall of flowers and fruit. On treatment of control and 50 ppm/tree auxin concentration, the percentage of fruit drop was 15.89% and 12.67%, higher than that of on 100 ppm/tree auxin concentration which was 8.84% (Table 2). The results of this study indicated that treatment of auxin was very important; because it could increase yields (number of fruits, fruit diameter, and weight of fruit per tree) and fruit quality such as weight per fruit, diameter of fruit, and total dissolved solids. The increased of total dissolved solids mean increased sweetness of the fruit and it was improving the selling price of the fruits.

4. Conclusion
The interaction between mycorrhizal inoculant dose and concentration of auxin hormone had no significant effect on all observed variables. Mycorrhizal inoculant dose of 100 g/tree increased yield and quality of fruits of Siam Orange reflected by higher number of fruit per tree (142.17 fruits), weight of the fruit per tree (13.53 kg), weight per fruit (96.00 g) and total dissolved solids (14.10 °brix) or increase of 44.21%; 57.87%; 11.89% and 21.03% than those of on controls. Auxin in the form of IBA concentration of 100 ppm/tree proven fruit drop and improve the quality of Siam orange fruits on off-season period, which was reflected by the higher number of fruit per tree (155.22 fruit), weight of fruit per tree (15.03 kg), weight per fruit (96.50 g) and total dissolved solids (14.43% brix) or increase of 52.01%; 55.61%, 18.17% and 29.41% than those of control.

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