

THE EFFECTS OF EFB COMPOST AND HEXACONAZOLE ON BETA-CAROTENE CONTENT AND ITS RELATIONSHIP WITH THE YIELD COMPONENTS OF SWEET POTATO VAR. VITATO

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Abstract

Field experiment was conducted on sandy tin-tailing soil to test the effectiveness of oil palm EFB compost and hexaconazole (HEX) growth regulator in increasing the storage root β -carotene content and yield components of sweet potato var. VitAto. The treatment plants were arranged using the RCBD replicated four times. The treatments used were the recommended rate of inorganic fertilizer practice in VitAto cultivation (control), EFB compost (F-EFB) and the combination of EFB compost with 10 ppm (F-EFB+10 ppm) and 30 ppm HEX (F-EFB+30 ppm). As results, F-EFB+30 ppm HEX treatment was the best in increasing the storage root β -carotene content up to 6052 $\mu\text{g}/100\text{ g}$, 15% significantly higher than control at the maturity stage. At similar growth stage, this treatment also increased the storage root total fresh weight, dry mass production and number by 35, 17 and 125% significantly higher than control respectively. The β -carotene content showed high correlation with these parameters ($r = 0.56^{***}$, 0.67^{***} and 0.53^{***} respectively) through correlation studies. In conclusion, F-EFB+30 ppm HEX was the best treatment applied with respect to the enhancement of storage root β -carotene content and yield components. It is proposed that this combination treatment could be used by farmers as an alternative practice to the inorganic fertilizer application in VitAto cultivation on sandy soil.

Keywords: β -carotene; sweet potato; VitAto; empty fruit bunch (EFB); hexaconazole (HEX)

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1. Introduction

1.1 Orange-fleshed sweet potato as cheap source for combating vitamin A deficiency

The VitAto variety is an orange-fleshed sweet potato (OFSP) that is high in β -carotene content which is a precursor of vitamin A. This variety was introduced by MARDI (Malaysian Agricultural Research and Development Institute) in 2007. The name VitAto is the combination of 'VitA' for vitamin A and 'to' for sweet potato (Tan *et al.*, 2010). The storage root can be consumed directly by boiling, steaming, frying or baking and can also be processed into food products such as doughnuts, muffins, buns, crispy cubes and candies (Tan *et al.*, 2010; Normah, 2010). The storage root and processed flour from the storage root of this crop also has a potential to replace imported potato tuber and wheat flour which Malaysia currently spent more than RM 60 million and RM 800 million per year respectively (Tan *et al.*, 2010).

The enhancement of β -carotene content in sweet potatoes were reported in many studies elsewhere, however, none for the local varieties such as VitAto. Although it has high storage root β -carotene content but it may reduce after processed into food product. If the content of β -carotene

can be increased at cultivating stage, the content may be preserved more after processed. The objective of this study was to investigate the effects of EFB compost and HEX and the combination of both treatments in enhancing the β -carotene content and yield components of VitAto storage root.

Beta-carotene is a major carotenoid compound in sweet potato (Woolfe, 1992; Hagenimana *et al.*, 1999a; Liu *et al.*, 2009). It is important to human health because it is a precursor of vitamin A (Desai, 2008; Tan *et al.*, 2010). The OFSP is categorized as food-based approach in combating vitamin A deficiency that is suitable for rural areas (Andrade *et al.*, 2009). This is due to the fact that the variety is a cheap source of vitamin A and is easily available to the poor as it can be easily grown throughout the year. The OFSP which serves as a staple food is better than most vegetables because it supplies significant amounts of both vitamin A and energy and therefore addressing both vitamin A deficiency and malnutrition (Andrade *et al.*, 2009).

1.2 The effects of fertilizer application on sweet potato storage root β -carotene content

The β -carotene content in sweet potato can be increased with N, P and K fertilizer applications which depend on soil type, fertilizer rates and crop cultivars (George *et al.*, 2002; Ukom *et al.*, 2009; Laurie *et al.*, 2012). Among the three nutrients, K is often associated with β -carotene content in plants (Lester *et al.*, 2010). The orange flesh sweet potato (OFSP) cultivars that are K deficient are generally have low carotene content in storage root (Lebot, 2009). The OFSP has higher concentrations of many nutrients including the nutrient K (Aywa *et al.*, 2013) which is also found in VitAto (Tan *et al.*, 2010).

The application of organic fertilizer probably can also increase the storage root β -carotene content. There is no study reported on the effects of EFB on the β -carotene content on any crops. However, the application of other organic fertilizers such as livestock manure (Koala *et al.*, 2013), pacesetter (Kareem, 2013), compost (type not stated) (Faber *et al.*, 2013) and broiler litter (Gichuhi *et al.*, 2014) were proven to increase the storage root β -carotene content in sweet potato.

1.3 The effects of hexaconazole on carotenoid in root crop species

Hexaconazole belongs to the triazole group of compounds. This compound has been shown to increase the levels of antioxidant and antioxidative enzymes, including plant carotenoid involved in scavenging over production of free radical reactive oxygen species (ROS) (Singh, 2005; Liu *et al.*, 2009; Gomathinayagam *et al.*, 2009). Carotenoid also is one of the naturally occurring plant pigments besides chlorophyll, betalains and anthocyanin (Zhou, 2012). In leaves, carotenoid was found in chloroplast but it was directly formed in chromoplast in the non-photosynthetic tissues of storage root such as carrot and sweet potato, (Desai, 2008; V. Lebot, personal communication, July 14, 2014). The HEX was shown to increase carotenoid content in the leaves and storage organ of carrot (Gopi *et al.*, 2007), *Plectranthus forskholii* Briq (Lakshmanan *et al.*, 2007), cassava (Gomathinayagam *et al.*, 2008), white yam (Jaleel *et al.*, 2008a), sweet potato (Sivakumar *et al.*, 2009), *Plectranthus aromaticus* and *Plectranthus vittiveroids* (Meena Rajalekshmi *et al.*, 2009). However, most of these studies were confined to the effects of HEX on general carotenoid but not specifically on the β -carotene content in storage root. Therefore, the current study was conducted to explore on the possibility of using HEX in increasing the β -carotene content in storage root of VitAto.

2. Materials and Methods

2.1 Experimental site and planting materials

The field experiment was conducted from March to July 2013 at MARDI Rawang station, Selangor (N3E16'17.89", E101E30'52.19"). The soil type was sandy tin tailing with 90.8% sand, 4.5% silt and 4.7% clay (Vimala, 2005). The monthly means for selected climatic factors were 24.1-28.6°C for temperature, 3.0-7.1 mm for rainfall and 76.2-83.4% for relative humidity during the experimental period (Malaysian Meteorological Department, 2014). The VitAto stem cuttings obtained from this station were used as planting materials.

2.2 Treatments

The recommended fertilizer practice for the cultivation of VitAto on sandy soil was used as a control treatment (MARDI, 2008). The second treatment was solely EFB compost (F-EFB) with nitrogen (N), phosphorus (P) and potassium (K) nutrient contents equivalent to the inorganic fertilizer nutrient content in the control treatment. The combination treatments between EFB compost with 10 or 30 ppm HEX designated as F-EFB+10 ppm (third treatment) and F-EFB+30 ppm HEX (fourth treatment), respectively.

2.3 Experimental design

The Randomized Complete Block Design (RCBD) was used as experimental design which replicated four times. The EFB compost with 10 and 30 ppm HEX were used based on their potential to give high yield as indicated on findings of earlier preliminary study. The EFB compost used was aGricare® Premium Compost (Myagri Eco-Biosciences Sdn. Bhd., Selangor, Malaysia) while the fungicide Anmi4.8SC (Advansia Sdn. Bhd., Selangor, Malaysia) was used as plant growth regulator with HEX as the main active ingredient. The formulation of this plant growth regulator was based on previous studies (Gopi *et al.*, 2007; Jaleel *et al.*, 2008b; Sivakumar *et al.*, 2009). The inorganic fertilizers for control and EFB compost were applied at 7, 28 and 35 days after planting (DAP) based on the VitAto production manual (MARDI, 2008). The application of HEX using drenching method was given at 20, 40 and 60 DAP.

2.4 Growth analysis procedure

The destructive growth analysis method and sequential growth analysis technique (Jolliffe *et al.*, 1982) were used to systematically sampling of plant samples by harvest and ease in field operation (Hunt, 1990). The storage root was separated from other plant parts after harvested at 55, 77 and 99 DAP. These sampling days are corresponding to storage root early and middle bulking and maturity stages respectively. After the storage root yield components parameters and storage root beta-carotene content were measured, the samples were oven dried at 72° C for 48 hours for dry mass determination.

2.5 Beta-carotene sampling procedure

The extraction of storage root β -carotene was carried out immediately after harvest since it was crucial to determine the level of β -carotene at that time of harvest to represent the actual content in relation to plant age. The sample used was not selected from any single or selected part of the storage root but instead include all available storage roots per plant to give an average content of

β -carotene per plant. This was carried out due to the uneven distribution of this compound, and it was reported that the highest concentration was found in the core (Wu *et al.*, 2008).

2.6 Beta-carotene analysis

The β -carotene extraction and determination method was carried out based on Rodriguez-Amaya and Kimura (2004). The β -carotene content of the sample was analysed using B13627 Unicam Helios alpha UV-VIS spectrophotometer (Severn Sales Limited, United Kingdom) by taking the absorbance value at 450 nm of wavelength after extraction. The absorbance values obtained from the spectrophotometer were used to calculate the total β -carotene content using the following formula (Rodriguez-Amaya and Kimura, 2004; Fikselová *et al.*, 2008):

$$\bullet \quad \text{Total } \beta\text{-carotene content } (\mu\text{g/g}) = \frac{A \times \text{Volume (mL)} \times 10^4}{(A_{1\text{cm}}^{1\%}) \times \text{Sample weight (g)}}$$

Where A is absorbance at 450 nm wavelength, volume is total volume of extract (50 or 25 ml), ($A_{1\text{cm}}^{1\%}$) is absorption coefficient of β -carotene in PE (2592). Multiply by 100 to give the β -carotene content in $\mu\text{g}/100\text{ g}$ of fresh storage root.

2.7 Storage root yield components

The yield parameters measured consisted of storage root and pencil size root numbers, fresh weight and storage root dry mass. The classification of storage root was based on Wilson and Lowe (1973) where, root diameter greater than 15 mm refer to the storage root and root between 5-15 mm refer to the pencil root. The pencil root was not considered as part of the yield but its diameter range was used to determine smaller storage root known as pencil size root. The difference in physical characteristics between both roots were identified and distinguished before the actual measurement and separation was carried out. In addition, the storage root dry mass production at the maturity stage were also used as yield component parameters. The storage root dry mass production was the sum of storage root and pencil size root.

2.8 Statistical analysis

The data was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparisons for separation of means. The relationship between variables was determined using Pearson correlation. The p values ≤ 0.05 were used to determine levels of significance.

3. Results

3.1 The effects of various empty fruit bunch compost with hexaconazole combination treatments on storage root beta-carotene content and the yield components

The β -carotene content generally increased from 55 to 77 DAP (bulking stages) before declined thereafter (Figure 1a). The β -carotene for F-EFB+30 ppm HEX was significantly higher than other treatment plants at the maturity stage but there was no significant difference at the previous stage.

The yield components such as storage root fresh weight, dry mass production and number generally increased from storage root bulking to maturity stages (Figure 1b, c and d respectively).

Most of these parameters were increased by F-EFB+30 ppm HEX at the harvestable stage significantly higher than control treatment. However, the pencil size root numbers and total fresh weight increased until bulking stage before declined thereafter (Figure 1e and f). No significant difference was observed for pencil size root numbers when reach maturity stage. In contrast, the storage root total fresh weight was increased by control treatment higher than F-EFB+10 ppm HEX at similar growing stage.

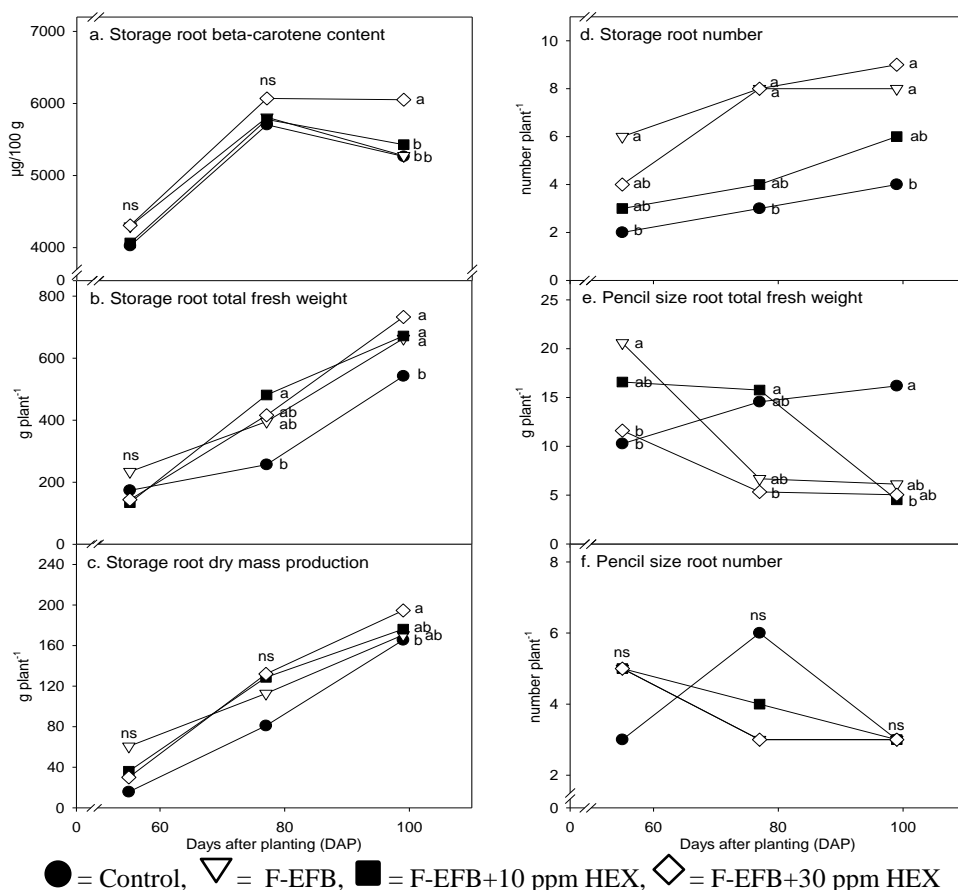


Figure 1(a-f): Storage root beta-carotene content (a) and yield components; (b-f) as affected by various EFB compost and HEX combination treatments at the specific growth stage (55, 77 and 99 DAP). Different letters follow different treatments at specific harvest time indicate a significant difference at $p \leq 0.05$ while ns indicate non-significant and the values are the means of four replicates

3.2 Correlation studies of various parameters related to storage root β -carotene content and yield components from the storage root bulking to maturity stage

The storage root β -carotene content was significantly correlated with most of the yield component parameters except for the pencil size root numbers and total fresh weight (Table 1). The highest correlation was with the storage root dry mass production, followed by the storage root total fresh weight and lastly storage root numbers ($r = 0.67^{***}$, 0.56^{***} and 0.53^{***} respectively).

Table 1. Correlation coefficient of various parameters related to storage root β -carotene content and yield components from the storage root bulking to maturity stages as affected by various EFB compost and HEX combination treatments.

Parameters	Storage root numbers	Storage root total fresh weight	Pencil size root numbers	Pencil size root total fresh weight	Storage root dry mass
Storage root β -carotene	0.53***	0.56***	-0.13	-0.31	0.67***

Note: *, **, *** indicate significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ respectively while ns indicate no significant correlation. The values are the means of four replicates

4. Discussions

4.1 Storage root beta-carotene content

4.1.1 General trend

The storage root β -carotene content responded positively to F-EFB+30 ppm HEX and significantly increased at maturity stage, higher than control. Therefore, the application of EFB compost should be supplemented with 30 ppm HEX to obtain better results. There is still lack of information on how EFB compost can increase β -carotene content in sweet potato storage root. However, other studies have shown the increment of carotenoid content in orange-flesh sweet potato when treated with various types of organic fertilizers. Organic treatments such as farmyard manure (Nedunchezhiyan *et al.*, 2010), well-decomposed livestock manure (Koala *et al.*, 2013) and broiler litter (Gichuhi *et al.*, 2014) were reported to significantly increase the carotenoid content in storage root when compared with untreated soil treatments. The usage of organic fertilizer called pacesetter (Kareem, 2013) and compost (type not stated) (Faber *et al.*, 2013) increased the content of storage root β -carotene as compared to the application of inorganic fertilizer. The HEX application was reported to increase the carotenoid content in the leaves of *Plectranthus forskholii* Briq (Lakshmanan *et al.*, 2007), cassava (Gomathinayagam *et al.*, 2008), *Plectranthus aromaticus* and *Plectranthus vettiveroids* (Meena Rajalekshmi *et al.*, 2009) and sweet potato (Sivakumar *et al.*, 2009). It was also proven to increase the carotenoid content in both the leaves and tuber of carrot (Gopi *et al.*, 2007) and white yam (Jaleel *et al.*, 2008a).

4.1.2 Improvement of beta-carotene content in storage root; fertilizer versus hormonal applications

The highest content of fresh storage root β -carotene at maturity stage was found in F-EFB+30 ppm treatment as much as 6052 $\mu\text{g}/100\text{ g}$ where it was 14.95% higher than F-MARDI. There is a broad range value of β -carotene content of storage root among the types and cultivars of orange-fleshed sweet potatoes. As reviewed from many studies, the storage root β -carotene content from various orange-fleshed sweet potato cultivars showed a range around 0.1 to 8.0 $\text{mg}/100\text{ g} \approx (100\text{ to }8000\ \mu\text{g}/100\text{ g})$ (Hagenimana *et al.*, 1999b; Hagenimana and Low, 2000), 802 to 1209 $\mu\text{g}/100\text{ g}$ (Williams *et al.*, 2013) and around 5091 to 16456 $\mu\text{g}/100\text{ g}$ (Laurie *et al.*, 2012). The famous superior cultivar like Resisto (Laurie *et al.*, 2012; Faber *et al.*, 2013) and Beauregard (Gichuhi *et al.*, 2014) showed high β -carotene content which could reach more than 20000 $\mu\text{g}/100\text{ g}$. If β -carotene content of storage root can be categorized into

several groups such as; a low range with content less than 1000, medium group with a range of 1000 to 10 000 and high with content greater than 10 000 expressed in $\mu\text{g}/100\text{ g}$, the VitAto cultivar could probably fall into the middle group.

4.2 The relationship between storage root beta-carotene content with yield components

4.2.1 Storage root versus pencil size root

The storage root β -carotene content generally showed significant positive correlation with storage root numbers and total fresh weight. It was in agreement with Wu *et al.* (2008) which showed positive relationship between storage root β -carotene content with the size of storage root. This relationship indicated that treatments that could increase storage root numbers and total fresh weight should also be able to increase storage root β -carotene content as shown by the combination treatments of F-EFB+30 ppm HEX. The storage root β -carotene content was negatively correlated with pencil size root (smaller storage root) numbers and total fresh weight which generally decrease with age from the bulking to maturity stages. Therefore, the treatments that supported the development of pencil size root could have contributed to low storage root β -carotene content as shown by F-MARDI (control).

The storage root total fresh weight (Figure 1b), dry mass production (Figure 1c) and number (Figure 1d), were increased with plant age from the storage root bulking to maturity stages. However, the level of storage root β -carotene content only increased from early to middle bulking stages (77 DAP) before declined thereafter which were experienced by all treatment plants. The result was in agreement with studies by Chattopadhyay *et al.* (2006) and Faber *et al.* (2013). In contrast, some other studies showed the trend of increment for both storage root yield components and storage root β -carotene content with plant age (K'osambo *et al.*, 1998; Hagenimana *et al.*, 1999b and Liu *et al.*, 2009).

4.2.2 Storage root size as a contributing factor to variation in beta-carotene content

The increase in the size of storage root to a certain extent could lead to a reduction in β -carotene content at the maturity stage. The carotene content seemed to have negative correlation with dry mass production (Wang, 1982 as cited in Lebot, 2009). The higher storage root fresh weight could contribute to lower carotene content and vice versa as reported in the study by Faber *et al.* (2013). However, Wu *et al.* (2008) stated that storage root β -carotene content has been shown to have a positive relationship with storage root size. The development pattern of β -carotene content itself is clearly explained in the varying results of β -carotene content. Like many other plant pigments, the development pattern of pigment production as well as pigment density is far from linear but rather sigmoidal. The pigment production and density may initially increase parallel to the increase in size of storage root, but later it remains constant reaching a plateau then begin to decline when pigments start to degenerate (G.E. Lester, personal communication, July, 7, 2012). It was postulated that the size of storage root in studies by Wang (1982); K'osambo *et al.*, 1998; Liu *et al.*, 2009 and Faber *et al.* (2013) probably did not reach the stage of β -carotene content declination as shown in this study. Therefore, the β -carotene content increased steadily parallel to the increase in the storage root size. The F-EFB+30 ppm HEX treatment was superior because it was able to retain β -carotene

content longer than other treatments during the bulking to maturity stages (Figure 1a). The β -carotene content of this treatment seemed to be retained longer during its maximum content period (plateau) and with only a slight reduction thereafter. It is concluded that, this combination treatment was better than a control treatment, single application of F-EFB and the combination between F-EFB+10 ppm HEX with respect to the enhancement of storage root β -carotene content.

4.2.3 The improvement of physiological traits to meet consumer preference

The storage root β -carotene content was significantly correlated with storage root dry mass production. It is well documented that the β -carotene compound in storage root can be preserved even after drying or processed into powder as flour (Tan *et al.*, 2010; Emmanuel *et al.*, 2012). Emmanuel *et al.* (2012) stated that about 92% of β -carotene compound remained in dried sweet potato chip and flour with only about 8% losses during processing. The VitAto flour still contained around 900 to 960 $\mu\text{g}/100\text{ g}$ of β -carotene content (Normah, 2010). There are two main objectives in growing sweet potato, which is firstly for the fresh market and secondly for the industrial market. For fresh market which is generally for human consumption, the high carotene content is highly preferred while for industrial market, high dry mass production with high dry mass content is more desirable (Lebot, 2009). It seemed that the application of EFB as organic fertilizer and HEX as plant growth regulator was shown to improve these parameters in VitAto since all these parameters gave positive response to treatments given.

5. Conclusion

The increment in β -carotene content could probably be due to an increase in storage root size. The higher the size of storage root the higher the β -carotene content. Both parameters exhibited similar trend which increased with plant age. However, when the size of storage root exceeded a certain size, the pigment content tended to decline, which could be due to the sigmoidal pattern of the increment. The most effective treatment that was able to increase storage root β -carotene content was EFB compost with 30 ppm HEX combination treatment. This treatment increased significantly higher β -carotene content than control and maintained higher β -carotene content throughout the maturity stage better than other treatments. It also increased most of the yield parameters such as storage root total fresh weight, dry mass production and number significantly higher than control at similar growth stage. Therefore, the F-EFB+30 ppm HEX may be the better option in increasing the yield quality as well as the yield components of VitAto cultivated on sandy soil.

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