EFFECTS OF CROP BIOLIFE ON THE GROWTH, FRUIT YIELD AND QUALITY OF STRAWBERRY (*FRAGARIA* × *ANANASSA*) IN A SUB-TROPICAL ENVIRONMENT

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ABSTRACT

Strawberry (Fragaria x ananassa) is a widely adapted small fruit grown from the low-altitude of tropics and subtropics to high-altitude in cold continental areas. Despite its nutritional benefits, little research has been undertaken to facilitate its wide scale production in sub-tropical environments. This experiment was laid down in a Randomised Complete Block Design (RCBD) with four replications. The aim was to determine the effects of different concentrations (0, 25, 50, 75 ppm) of Crop Biolife (CB) on growth, yield and quality of strawberry. The results revealed that plants treated with 75 ppm CB significantly (P<0.05) had more growth in number of leaves, petiole length, plant spread and leaf area. The highest leaf nitrogen (2.63%) and chlorophyll content (32.65) were observed in strawberry plants treated with 75 ppm CB. Control plants sprayed with distilled water took fewer days to produce first flower and fruit as compared to CB treated plants at 75 ppm which later produced more flowers and fruits compared to the control plants. Fruit weight and yield also increased with increasing CB concentrations with 75 ppm having the highest results. Titratable acidity of the fruits was significantly (P<0.05) higher at 75 ppm (0.82%) with less vitamin C (50.50 mg/g) and less total soluble solids (6.10°Brix). Results from CB treated plants at 75 ppm were the best in this experiment but more research needs to be done in the open field as this one was done in a lath house with plants grown in containers.

Keywords: **S**trawberry, crop biolife, yield, quality, subtropical environment

INTRODUCTION

The cultivated strawberry is a hybrid plant between two American species, *Fragaria chiloensis* of western North and South America and *Fragaria*

virginiana of eastern North America (Lolaei *et al*., 2012). The commonly cultivated strawberry is *Fragaria* × *ananassa*. The hybridization of the two species occurred around 1850 in France and hundreds of varieties have been selected and named since then. Although other *Fragaria* species are also cultivated, this hybrid is the primary source of commercially produced strawberries. Strawberries are very nutritious fruits and excellent sources of vitamin C, antioxidants and flavonoids. The strawberry fruit is very low in calories, saturated fat, cholesterol and sodium. It is also a good source of folate and potassium, and a very good source of dietary fiber, and manganese and ellagic acid, a nutraceutical that has powerful anti-oxidant and anti-carcinogen properties (Thiele, 1986).

Crop Biolife (Aussau Laboratories PTY LTD, New South Wales, Australia), a mixture of bitter-orange extracts; contains naturally occurring flavonoids that provide benefits beyond nutrient uptake by stimulating the biosynthetic pathway of the plants, hence improving their overall health (van der Westhuizen, 2016). Once applied on the plant leaves, the flavonoids enter the leaf structure through the stomata and create natural triggers to plant health through biosynthesis; they also stimulate the already existing flavonoids in plants which lead to improved plant health. Some benefits of Crop Biolife include improved fruit colour, improved root health, nodulation, and exudation; improved soil biology, better fruit set and higher brix levels in fruits (van der Westhuizen, 2016). It has also been reported to act on the plant through its growth regulation activity, enhancing cell division, cell elongation and accumulation of cell metabolites. Farmers from California reported an increase in brix of grapes (11 – 13 $^{\circ}$B) and some improved colour on green tomatoes (36 – 23%) (van der Westhuizen, 2016).

Commercial strawberries are successfully grown in a broad range of climates including temperate, grassland, Mediterranean and subtropical regions (Hancock, 2000), however, production of strawberries in the sub-tropics is not uniform due to poor winter chilling (Darnell *et al*., 2003), which results in poor flower initiation, poor fruiting and low total yields. There is a need to find plant bioregulators which have been used in research trials for strawberry production in the temperate regions and had shown to have a positive effect on growth and yield. The objective of this study was to determine the effects of crop Biolife applications on the growth, fruit yield and quality of strawberry fruits in Swaziland.

MATERIALS AND METHODS

Experimental site

The research was carried out at the University of Swaziland (UNISWA), Faculty of Agriculture, Luyengo Campus, at the Horticulture Department Farm lath house (black 50% netshade; Mahalaxmi, India) between September 2014 and February 2015. The farm is located at Luyengo, Manzini Region, in the Middleveld agro-ecological zone, 26'0 32' S – 31'0 14' E (Murdock, 1970), and the average altitude of this area is 750 m above sea level. The annual mean precipitation is 980 mm with most of the rain falling between October and March (MOAC, 2004).

Experimental design

The research was done using the garden strawberry runners and they were obtained from a local farmer. Strawberry runners were planted in a potting media comprising of sand, soil and compost mixture in a 1:1:1 ratio, in 3.5 liter polythene bags. The bags were spaced at 30 cm by 35 cm in the lath house. Crop Biolife (CB) was used as the treatment with three concentrations of 25, 50, 75 ppm and distilled water was used as the control treatment. The experiment was laid down in a Randomised Complete Block Design (RCBD) with four replications. The plants were watered thrice weekly depending on the weather and weeds were removed occasionally.

Treatment preparations

One-litre solutions of Crop biolife were prepared by adding 25, 50 and 75 ppm concentrations CB (5-10%, black/brown liquid, Biorevolution, Cape Town, South Africa) in conical flasks and topped them with distilled water. All the treatments were applied by spraying using a five-litre knapsack sprayer to a runoff onto the plant leaves two weeks after transplanting and sprayed again after another two weeks.

Vegetative growth parameters

Plant growth parameters for strawberry were measured from (four plants per replicate) randomly selected and tagged plants, at intervals of 2, 4, 6 8 and/or 10 weeks after transplanting (WAT) during the experimental period. Leaf number was counted using the triplicate leaves per randomly tagged plant per treatment and the total number was recorded. The leaf petiole

length and plant spread were measured using a 30 cm-ruler per tagged plant per treatment concentration; the total number was recorded. The number of runners and the runner plants were recorded for the various treatments. Leaf area was measured by taking leaf length and breadth using a 30 cm ruler for all the treatments. The measurements were multiplied together with a correction factor (L x W x 0.75) (Edje and Ossom, 2009). Leaf nitrogen (%) was determined through the digestion and distillation processes (AOAC, 1990). Chlorophyll content index was measured from randomly selected plant leaves using a chlorophyll content meter (CCM-200, Opti-Sciences; Chicago, Illinois, USA) which gives a chlorophyll content index (CCI) value as an estimate for leaf chlorophyll. Leaves were placed in-between the CCM lever and a CCI value was displayed on the screen and recorded; readings were taken fortnightly from 4 to 10 WAT.

Fruit parameters

Days to first flowering and fruit formation, and the number of flowers and fruits per plant were recorded for all the treatments. Fruits were weighed using a balance (HCB602H, Adam Equipment, Johannesburg, South Africa). A refractometer (Master.53T Brix~53%, Tokyo, Japan) was used to measure the total soluble solids in the fruits. Fruit juice was extracted by squeezing the fruit with hands, a drop of the juice was put on the prism which was then closed to allow taking of the readings. Fruit yield was calculated by multiplying the number of flowers, number of fruits and the fruit weight per concentration and treatment. Titratable acidity in fruits was measured by firstly weighing 50 g of fruits for the treatments using procedures from AOAC (1990). Ascorbic acid was determined using the procedures from the University of Canterbury (2014).

Data analysis

Collected data was subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS, 1990). Means, where statistical differences were detected, were separated using Duncan's New Multiple Range Test (DNMRT) at P=0.05 (Gomez and Gomez, 1984).

RESULTS

Experimental site

The research was carried out at the University of Swaziland (UNISWA), Faculty of Agriculture, Luyengo Campus, at the Horticulture Department Farm lathhouse (black 75% netshade; Mahalaxmi, India) between September 2014 and February 2015. The farm is located at Luyengo, Manzini Region, in the Middleveld agro-ecological zone, 26'0 32' S – 31'0 14' E (Murdock, 1970), and the average altitude of this area is 750 m above sea level. The annual mean precipitation is 980 mm with most of the rain falling between October and March (MOAC, 2004).

Temperature and relative humidity

The average temperatures and relative humidity during the experimental study are shown below in Table 1.

Table 1: Average temperatures and relative humidity

Month	**Temperature (°C)**		**Relative Humidity (%)**	
	Minimum	Maximum	Morning	Afternoon
September	15	28	70	49
October	14	24	87	66
November	17	26	85	73
December	17	26	89	71
January	17	28	83	87
February	17	35	89	74

Vegetative growth

Leaf number of strawberry plants was significantly ($P<0.05$) different in all the treated plants. Plants sprayed with Crop Biolife at 75 ppm significantly produced more leaves (Figure 1) than the other concentrations and control plants. Leaf number increased for all concentrations with an increased in the number of weeks after transplanting. The highest number of leaves in all concentrations was observed at 8 WAT. Long petioles were observed in plants treated with 75 ppm of CB (Figure 2) at 10 WAT for all treatments

while the lowest leaf petioles was observed at 4 WAT; the trend in petiole length was similar to that of leaf number.

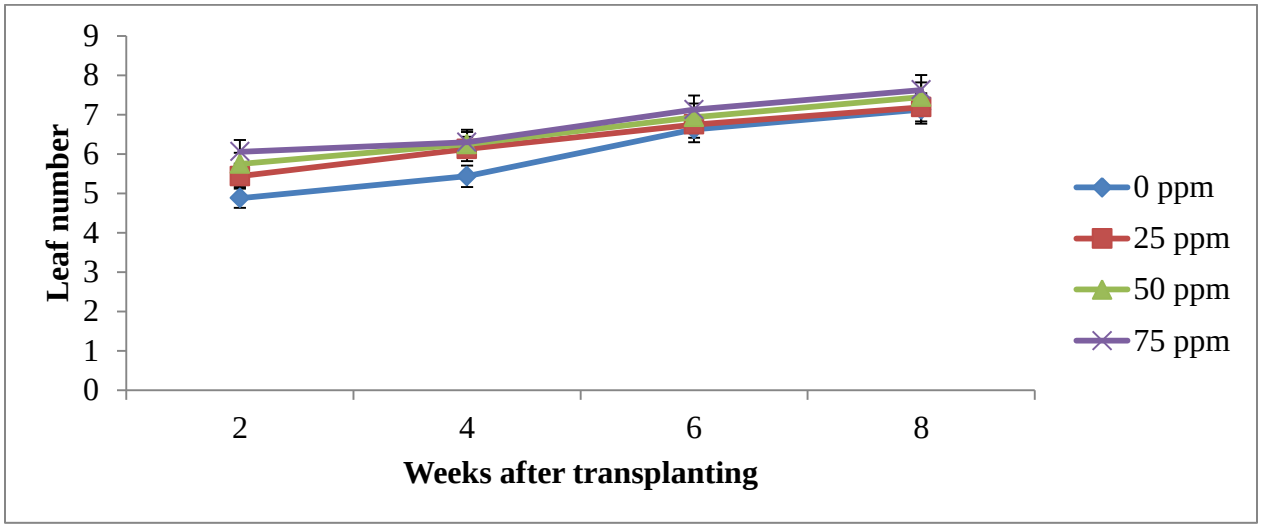

Figure 1. Effects of Crop Biolife on leaf number in strawberry. Bars are standard error (S.E.) below and above the mean

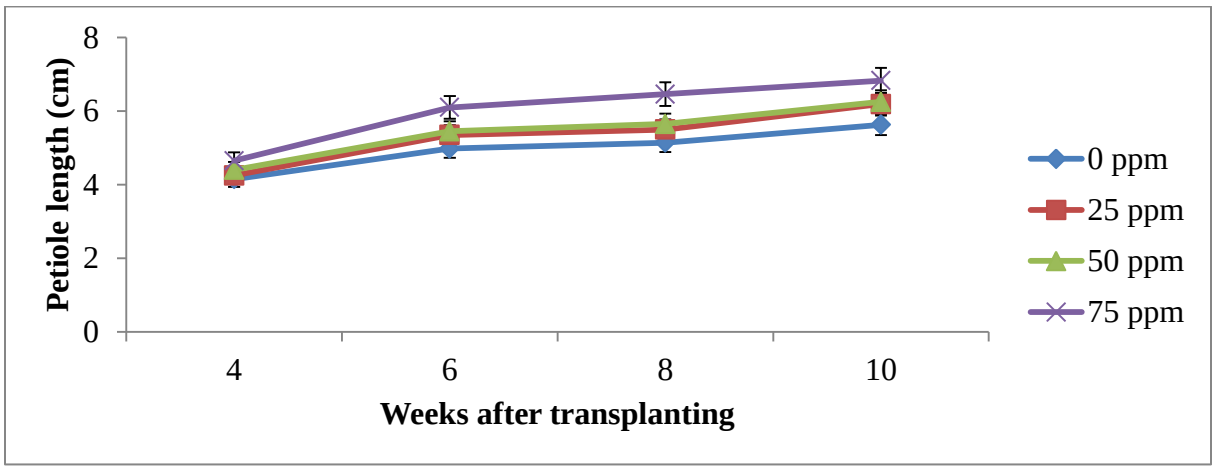

Figure 2. Effects of Crop Biolife on petiole length in strawberry. Bars are standard error (S.E.) below and above the mean

Significant differences ($P<0.05$) were observed for plant spread and leaf area in all the treated plants. Plant spread increased for all the concentrations with the number of weeks after transplanting with 10 weeks having the highest plant spread. Crop Biolife treated plants at 75 ppm had the best plant spread followed by 50, 25 ppm and control plants; the increase in plant spread was observed with every increase in concentration

of CB (Figure 3). Plants sprayed with CB at 75 ppm had significantly ($P<0.05$) higher leaf area compared to the other treated plants (Figure 4).

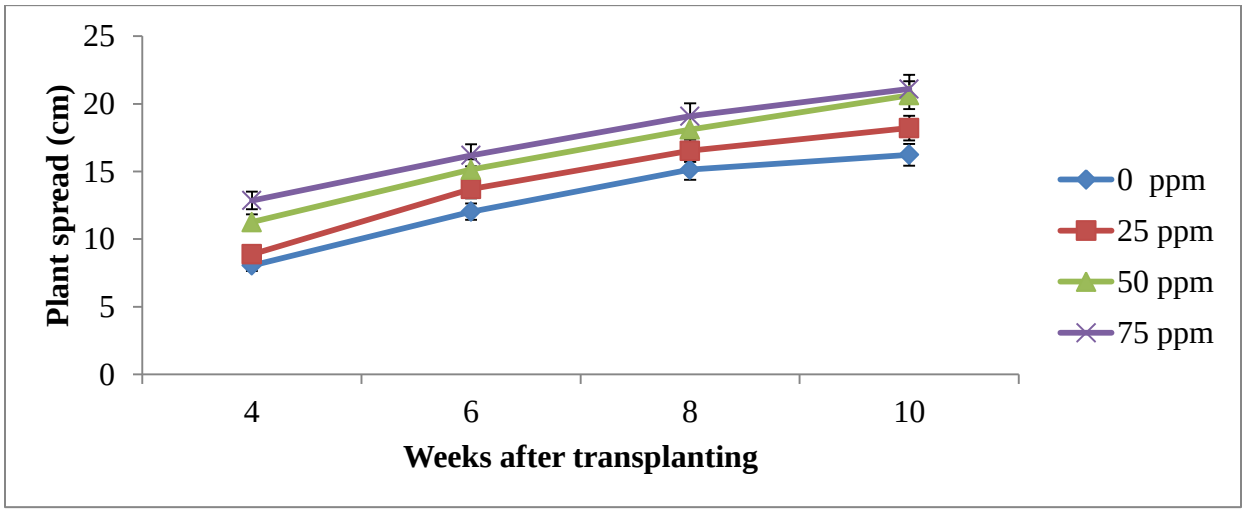

Figure 3: Effects of Crop Biolife on plant spread in strawberry. Bars are standard error (S.E.) below and above the mean

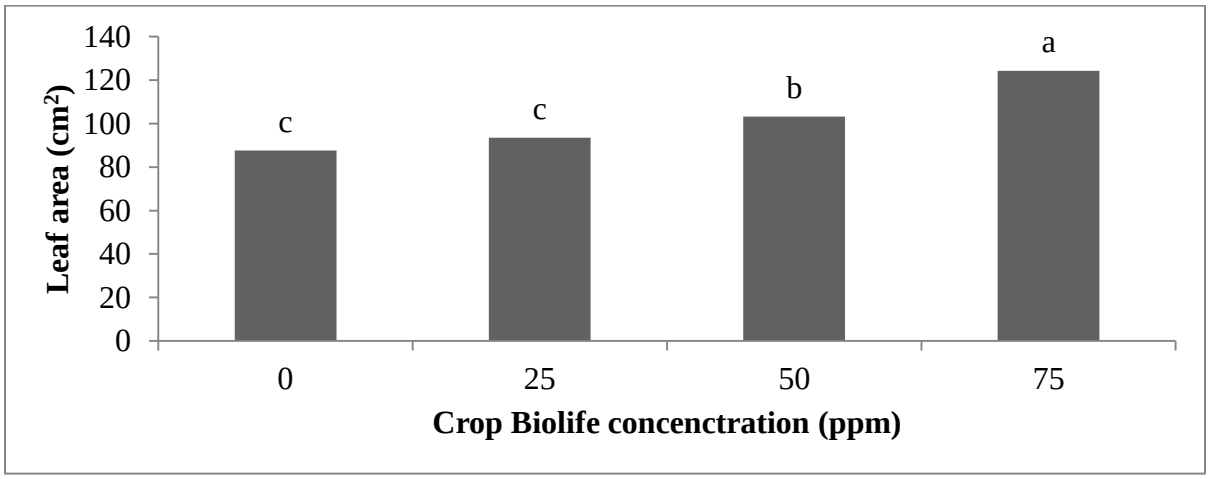

Figure 4: Effects of Crop Biolife on leaf area in strawberry. Bars followed by same letter not significantly different. Mean separation by DNWRT at P=0.005.

Nitrogen and chlorophyll contents

Both percentage leaf nitrogen (N) and chlorophyll content index (CCI) were significantly ($P<0.05$) different with increasing amounts for CB treated plants. Plants treated with CB at 75 ppm had the highest leaf nitrogen (2.63%) at ten weeks after transplanting (Figure 5) followed by 50, 25 ppm and control plants; which then decreased at 15 WAT. The same trend was

also observed for CCI. Chlorophyll content index was highest (32.69) in CB treated plants (Figure 6) at 75 ppm at week ten. Both leaf N and CCI increased with an increase in concentrations for all the CB treated plants.

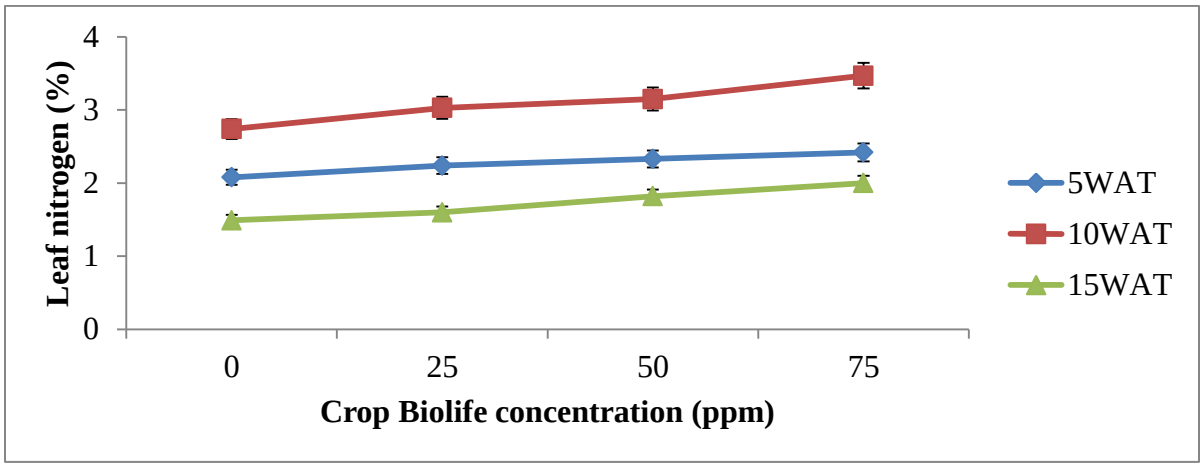

Figure 5. Effects of a Crop Biolife on leaf N Content in strawberry. Bars are standard error (S.E.) below and above the mean

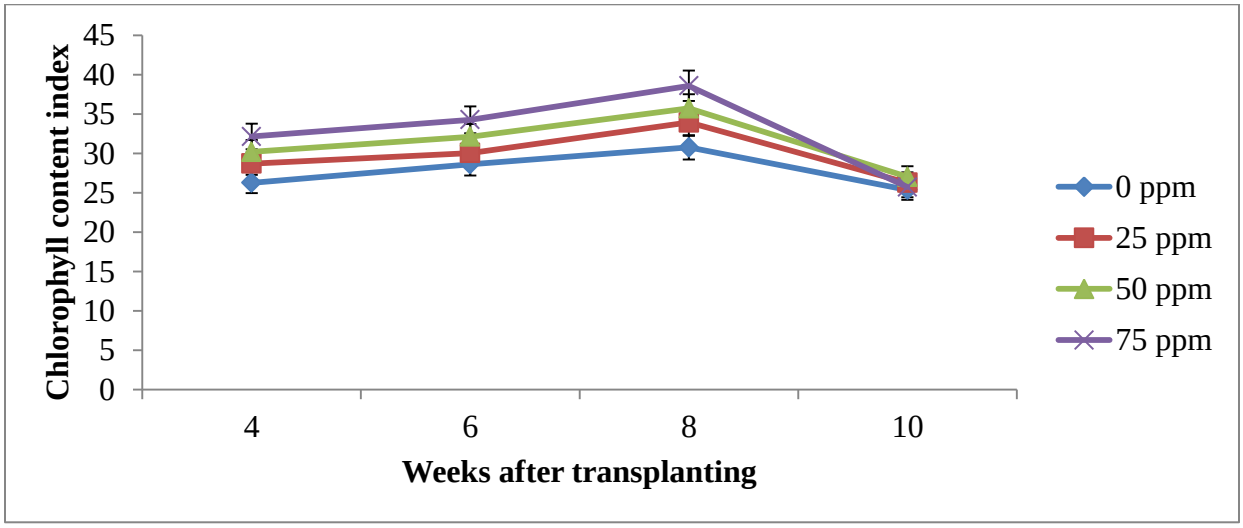

Figure 6. Effects of Crop Biolife on chlorophyll content index in leaves of strawberry. Bars are standard error (S.E.) below and above the mean

Reproductive growth

There were significant ($P<0.05$) differences in treatment concentrations for the number of days to first flowering and fruit formation; and the number of flowers and fruits formed. The number of days to first flowering and fruiting were increasing with an increase in concentrations for CB treated plants

(Figure 7). The CB treated plants took more days at 75 ppm to start producing flowers (64.25 days) and fruits (73 days). Plants sprayed at 75 ppm with CB produced more flowers (14.69) and fruits (13.06) (Figure 8). Both flower and fruit numbers increased with increasing concentrations of CB, but fruit numbers were less than the flower numbers.

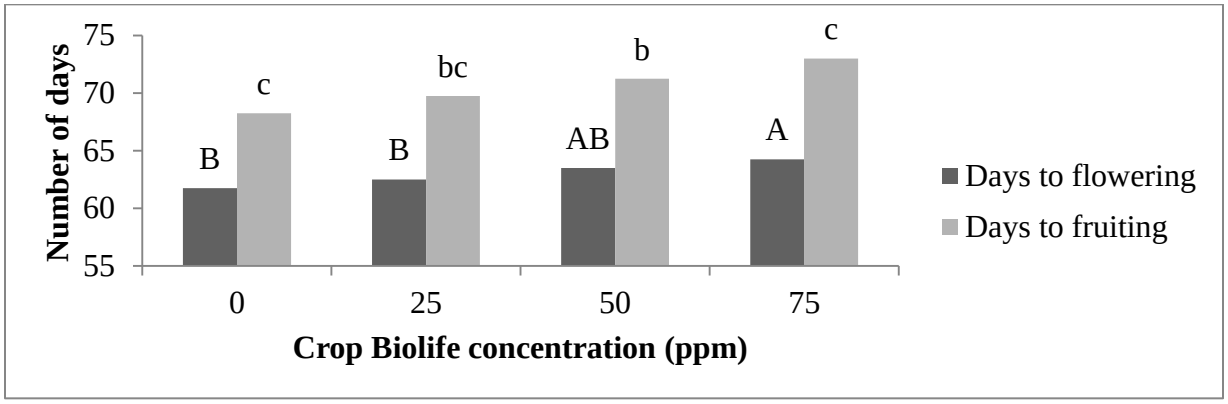

Figure 7. Effects of Crop Biolife on the number of days to first flowering and fruiting in strawberry. Bars for each parameter followed by same letter not significantly different

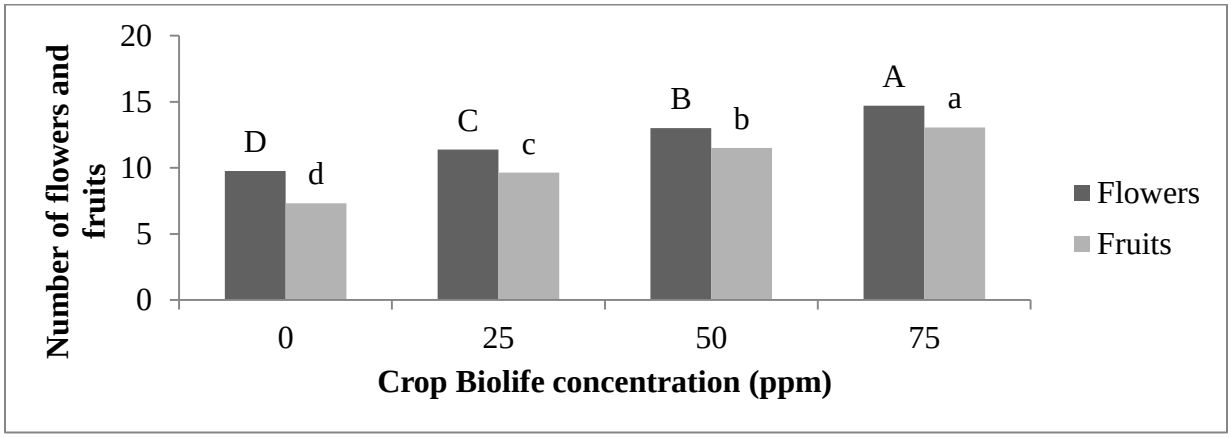

Figure 8. Effects of Crop Biolife on the number of flowers and fruits in strawberry. Bars for each parameter followed by same letter not significantly different

Fruit weight increased with each increase in the concentration of CB, with control fruits having the least weights. Fruits from plants treated with CB had higher weight (4.16 g) at 75 ppm (Figure 9) as compared to those from 25 and 50 ppm treated plants. Yield from CB treated plants was significantly higher at 791.67 g per plant at 75 ppm (Figure 10) followed by 50, 25 ppm

and control treated plants. Fruit yield was directly associated with the number of plant leaves, leaf area, number of flowers and fruits produced and the fruit weight.

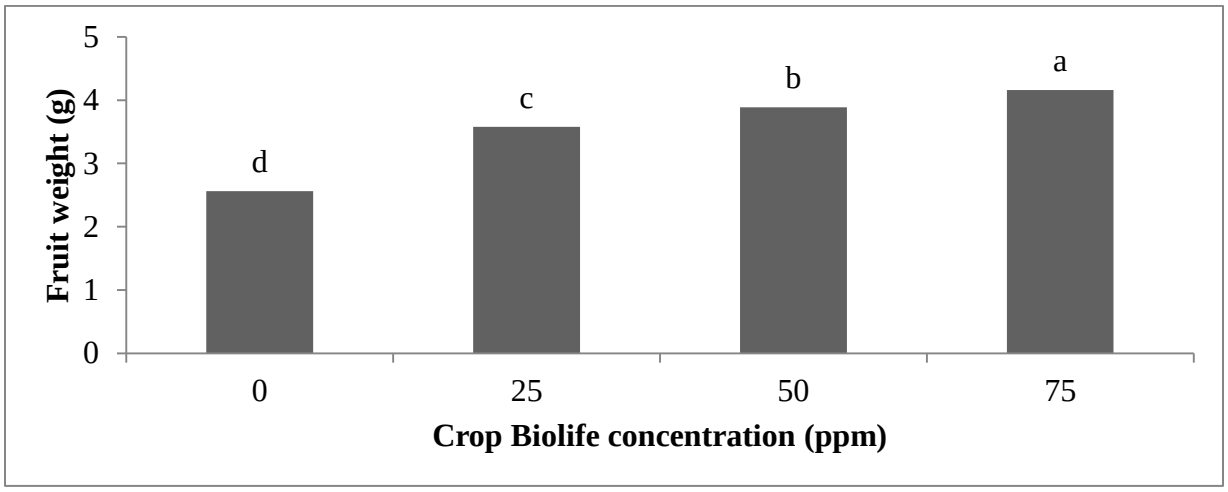

Figure 9. Effects of Crop Biolife on fruit weight in strawberry. Bars followed by different letter significantly different

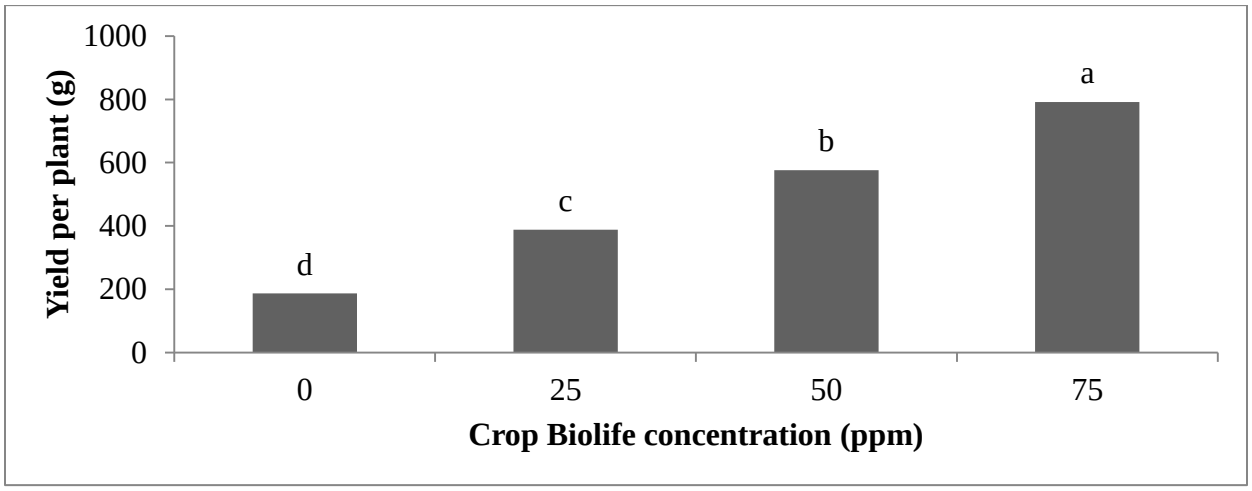

Figure 10. Effects of Crop Biolife on fruit yield per plant in strawberry. Bars followed by different letter significantly different

Fruit quality

Significant differences ($P<0.05$) were observed in total soluble solids, fruits' titratable acidity and vitamin C content in all treatment concentrations. Total soluble solids for CB fruits decreased with each increase in CB concentration (Figure 11), while their titratable acidity increased with increasing CB

concentrations increased (Figure 12). Fruits from CB treated plants had less total soluble solids and more titratable acidity (Figures 11 and 12). Fruits from plants treated with CB had more acidity (0.82%) at 75 ppm. Vitamin C content in fruits from CB treated plants decreased with each increase in concentrations of CB (Figure 13).

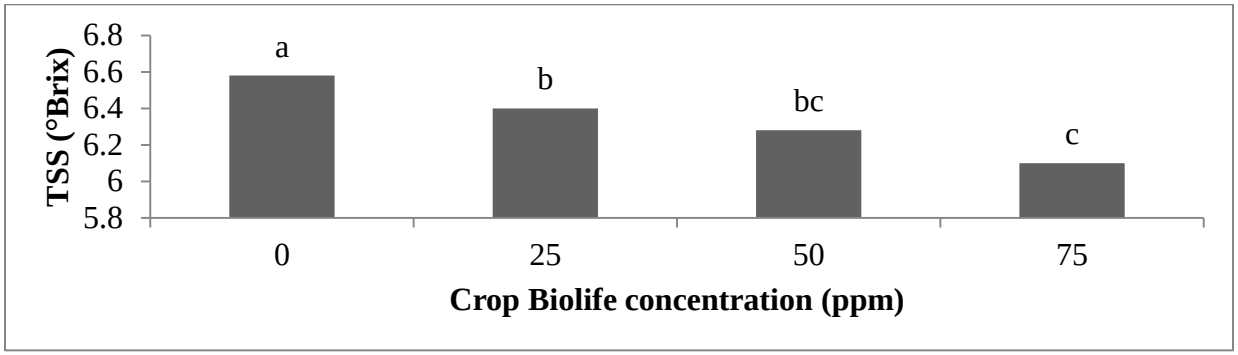

Figure 11. Effects of Crop Biolife on the TSS of fruits of strawberry. Bars followed by same letter not significantly different

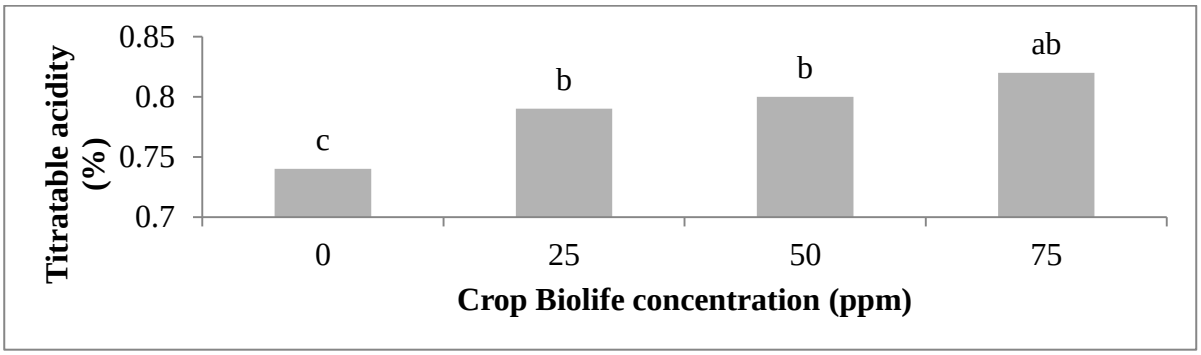

Figure 12. Effects of Crop Biolife on the titratable acidity of fruits in strawberry. Bars followed by same letter not significantly different

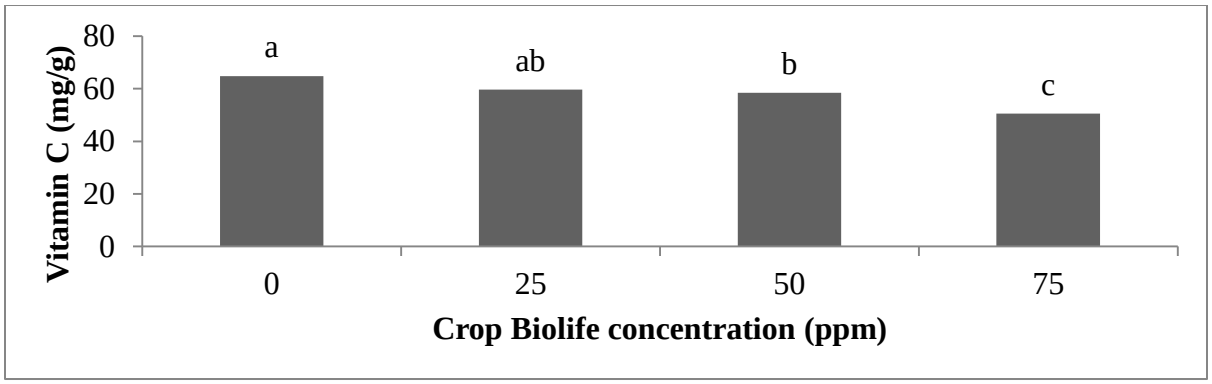

Figure 13. Effects of Crop Biolife on the vitamin C of fruits in strawberry. Bars followed by same letter not significantly different

DISCUSSION

The increase in the number of leaves per plant sprayed with CB could possibly be ascribed to the fact that nitrogen often increases plant growth and height and this resulted in more nodes and internodes and subsequently more production of leaves. Control plants had the least plant spread due to their small leaf petioles and numbers and the slow leaf elongation rate; this resulted in a less leaf area. A decrease in nitrate supply, especially for control plants, can lead to a large reduction in leaf-elongation rate and a decrease in final leaf size due to a smaller final cell size (Santos and Chandler, 2009). Increased nitrogen quantities enhanced growth and consequently influenced leaf elongation, expansion and development hence leading to a better plant spread and leaf area for CB treated plants.

In this study, CB sprayed plants had more nitrogen and chlorophyll content with increasing concentrations. This may be due to the higher nitrogen content of CB. The CB sprayed plants containing highest leaf N also contained the highest chlorophyll content. This could have been due to the fact that nitrogen is contained in the chloroplasts which also contain chlorophyll, as a result more chloroplasts would mean more chlorophyll and nitrogen. Plants contain 1 to 3% nitrogen on a dry weight basis, around 70% of leaf N is contained in the chloroplasts hence leading to yellowing or pale colours when it is deficient (Taiz and Zeiger, 2006). Peoples and Dalling (1988) also reported that up to 75% of nitrogen in mesophyll cells can be located in the chloroplast and similar relationships have been observed between chlorophyll content index and foliar or whole plant nitrogen in agricultural species, including maize, cotton and grasses. Nitrogen values ranged from 1 to 3%, which are within reported ranges for strawberry leaf tissues (Casteel, 2011). Peng *et al*. (1993) found that most within-species variation in relationships between chlorophyll content and N could be explained by differences in leaf thickness and how they perceive light; these relationships can be improved by calculating specific leaf weight (Peng *et al*., 1993) or considering N on an area, rather than dry weight basis. The results indicated that leaf N and chlorophyll contents of strawberry cultivars showed great variability but in a linear pattern which can probably be used to conclude that chlorophyll provides a linear approximation of total N.

Chlorophyll meter values are based on light absorption by the leaf chlorophyll at specific spectral bands and they may be affected by the

degree of yellowness or greenness of the leaf. Chlorophyll content has been used as a direct indication of plant health and condition. It can also be used to manage nutrient optimization programs that both improve crop yield and help protect the environment (Casteel, 2011). Changes in chlorophyll content can occur as a result of nutrient deficiencies, exposure to environmental stress, and exposure to certain herbicides and differences in light environment during growth (shading).

Strawberries require cold temperatures to initiate flowering but this can be bypassed by use of plant regulators in hot environments hence reducing the time needed before flower induction and fruit formation. Crop biolife treated plants took more days to produce the first flower and fruit; this could be associated with the fact that nitrogen may have indirectly affected flower induction by increasing the vigour of the whole plants. Plants took more time growing vegetatively under unfavorable growing conditions for flower induction as they were grown during the hot days even though they are short day plants. During their vegetative stage, the CB treated plants probably manufactured more food which was used in producing most of the inflorescence as it was seen in treatments with more CB concentration applied. Motamedi *et al*. (2013) explained that an enhancement of N quantity has shown increment in the inflorescence and flower number and fruit yield of strawberries. Fruits produced in all the treatment concentrations were less than the number of flowers; this might have been due to flower abortion, drop and/or fruit drop.

The increase in fruit weight for all the treated plants could possibly have been due to increasing cell size and/or cell numbers. Reserved plant assimilates from the vegetative stage of the CB treated plants were used towards fruit development as more sink capacity resulted in more assimilates being allocated to the source (fruit) hence the increase in weight (Singh and Singh, 2006). More fruit weight in CB treated plants was directly associated with high leaf nitrogen content as there were more assimilates during fruit development; more leaf N led to high fruit weight.

Higher yield might have been due to formation of more metabolites by large and more leaves in the CB treated plants resulting in bumper flowering, fruit setting besides better vegetative growth. The increase in fruit yield due to CB application observed in the current study was attributed to the enhanced vegetative growth (leaf area and leaf number per plant), dry

matter accumulation and increase in yield components (number of flowers and fruits, and fruit weight) (Huang and Huang, 2005). Singh and Singh (2006) explained that higher yields are expected from plants which had more N during their growth as N is made available to the plants roots and translocated to the flowers through plants foliage. It is during fruit ripening when the carbohydrates reserves in the roots and plant stem are drawn upon heavily and hydrolysed into sugars. Yield capacity of strawberry plants can be influenced by more complex environmental factors other than flowering. Chilling effect as a temperature regulation method is indirect and mediated through flowering time, carbohydrate reserve, vegetative viguor (Darnell *et al*., 2003) and photoperiod (Hytonen *et al*., 2004).

Fruits from CB treated plants had higher weights compared to those from control plants and they were associated with less total soluble solids as they contain more water which probably dissolved the solids or sugars. Large berries tend to have more water content than sugar content (Faust, 1989) and this is because the water in the large fleshy part dilutes out the little sugar in the small rind surfaces (Pritts, 1998). Results from Motamedi *et al*. (2013) have shown that nitrogen-based applications to strawberry reduce the fruits' total soluble solids and result in fruits with a lower titratable acidity, which contradicts results from this study. Al-Jamali and Al-Wardi (2010) also found that sprays with ethephon and Crop biolife-B maintained the good taste of the strawberry fruits as it was indicated by their unchanged main taste components, total soluble solids and titratable acidity. The differences in the above mentioned results might be due to the different cultivars used in the studies. Bell *et al*. (1979) determined that a nitrogen fertilisation rate of 114 kg/ha improved fermentation rates, concentration of esters, and wine quality of grape (*Vitis vinifera* L.) in comparison with lower N rates. Favell (1998) concluded that titratable acidity which is mainly citric acid can be used as an appropriate marker for monitoring quality changes in fruits and vegetables during transportation, storage and processing.

Fruits from CB treated plants had low vitamin C contents and this could be associated with their leaf N which was higher than in other treatments. High N contents are known to decrease vitamin C contents in many fruits and vegetables as they increase plant foliage and reduce light intensity and accumulation of vitamin C in shaded plant parts (Lee and Kader, 2000). In a study by Nagy (1980), it was observed that there were

reduced levels of vitamin C in juices of oranges, lemons, grapefruits and mandarins due to the application of high levels of nitrogen fertiliser. Ascorbic acid is also in high contents in immature fruits as it was in our control fruits. Nagy (1980) reported that immature citrus fruits contained the highest concentration of vitamin C whereas ripe fruits contained the least.

CONCLUSIONS

Plant bioregulators can be used to improve growth and production of strawberry in a sub-tropical environment. Crop Biolife applications led to increased vegetative and reproductive growth of strawberry. Vegetative growth increased with increasing Crop Biolife concentrations, with 75 ppm giving the best results. Fruits were significantly bigger than those from the other concentrations which might be a result of the plants having more nitrogen and chlorophyll contents; the fruits had more titratable acidity while vitamin C and total soluble solids contents were low.

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LITERATURE CITED

Al-Jamali, A.F. and Al-Wardi, S.S. (2010). Croplife-B and ethephon: Candidates for use in strawberry sustainable organic horticulture. *Journal of Food, Agriculture and Environment.* 8 (3&4): 683-688.

Association of Official Analytical Chemists. (1990). *Official Methods of Analysis*.15th edition. Association of Official Analytical Chemists, Washington, D.C., USA.

Bell, A.A., Ough, C.S. and Kliewer, W.M. (1979). Effects on must and wine composition, rates of fermentations, and wine quality of nitrogen fertilization of *Vitis vinifera* var. Thompson seedless grapevines. *American Journal of Enology and Viticulture.* 30: 124–129.

Casteel, S. (2011). Strawberry Fertility and Nutrient Management, NCDA&CS Agronomic Division. http://strawberries.ces.ncsu.edu/wp-content/uploads/2014/10/7-FertilityCombined.pdf. 10/07/14.

Darnell, R.L., Cantliffe, D.J., Kirschbaum, D.S. and Chandler, C.K. (2003). The physiology of flowering in strawberry. *Horticultural Reviews (American Society of Horticultural Science.* 28: 325–349*.*

Edje, T.O. and Ossom, E.M. (2009). *Crop Science Handbook*. Blue Moon Publishers, Manzini, Swaziland, pp. 179.

Faust, M. (1989). *Physiology of Temperate-zone Fruit Trees*. Wiley-Interscience. New York, New York. USA.

Favell, D.J. (1998). A comparison of the vitamin C content of fresh and frozen vegetables. *Food Chemistry.* 62: 59-64.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedure for Agricultural Research. 2nd Edition. John Wiley and Sons, Singapore.

Hancock, J.F. (2000). Strawberries. *In*: Temperate Fruit Crops in Warm Climates. Erez, A. (Eds.) Kluwer Academic Publishers, Netherlands.

Huang, J. H. and Huang, L. (2005). The application of GA_3 in citrus orchards. *South China Fruits.* 3: 32-36*.*

Hytonen, T., Palonen, P., Mouhu, K., Junttila, O. (2004). Crown branching and cropping potential in strawberry (*Fragaria* × *ananassa* Duch.) can be enhanced by day length treatments. *Journal of Horticultural Science and Biotechnology.* 79: 466–471.

Lee, S.K. and Kader, A.A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology.* 20: 207–220.

Lolaei, A., Behzad, K., Raad, M.K., Rezaei, M.A. and Maghsoudi, M. (2012). Effect of paclobutrazol and salinity on vegetative and sexual growth and fruit quality of Strawberry (*Fragaria* × *Ananassa* Duch. cv. Selva). *Annals of Biological Research.* 3 (10): 4663-4667.

Ministry of Agriculture and Cooperatives (MOAC). (2004). Country report on state of animal genetic resources in Swaziland: A contribution to the first report on the state of the world's animal genetic resources. http://webache.googleusercontent.com/search?q =cache:JQE-YqpuSb0J:ftp://ftp.fao/ 010/a1250e/annexes/CountryReports/Swaziland.pdf 04/02/14.

Motamedi, S., Jafarpour, M. and Shams, J. (2013). Evaluation of nutrition on flower number and yield of strawberry in greenhouse. *International Journal of Agriculture and Crop Sciences.* 5 (18): 2091-2095.

Murdock, G. (1970). Soils and land capability in Swaziland. Ministry of Agriculture and Cooperatives, Mbabane, Swaziland.

Nagy, S. (1980). Vitamin C contents of citrus fruit and their products: a review. *Journal of Agriculture and Food Chemistry.* 28: 8–18.

Peng, S., Garcia, F.V., Laza, R.C. and Cassman, K.G. (1993). Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. *Agronomy Journal.* 85: 987–990.

Peoples, M.B. and Dalling, M.J. (1988). The interplay between proteolysis and amino acid metabolism during senescence and nitrogen reallocation. *In*: Nooden, L. D. and Leopold, A. C. (Eds.), Senescence and Aging in Plants. Academic Press, San Diego, CA, USA.

Pritts, M. (1998). Strawberry nutrition. Department of Pomology, Cornell University. Ithaca, New York, USA.

Santos, B.M. and Chandler, C.K. (2009). Influence of Nitrogen Fertilization Rates on the Performance of Strawberry Cultivars. *International Journal of Fruit Science.* 9 (2): 126-130.

Singh, A. and Singh, J.N. (2006). Studies on influence of biofertilizers and bioregulators on flowering, yield and fruit quality of strawberry cv. sweet Charlie. *Annals of Agricultural Research New series.* 27 (3): 261-264.

Statistical Analysis System (SAS). (1990). Statistical Analysis System Institute Inc., SAS users' guide: Statistics, 5th edition, SAS Institute Inc., Cary, North Carolina, USA.

Taiz, L. and Zeiger, E. (2006). Plant Physiology. Sinauer Associates Inc. Publishers, Sunderland, Massachusetts, USA.

Thiele, G. (1986). Strawberries. pp. 219-222. *In*: Temperate and Subtropical Fruit Production. (Eds.) Jackson, D. Wright and Carman Ltd., New Zealand.

University of Canterbury. (2014). Determination of Vitamin C Concentration by Titration. College of Science, University of Canterbury, New Zealand. http://www.outreach. canterbury.ac.nz. 12/10/14.

van der Westhuizen, P. (2016). Cropbiolfe. http://biorevolution.co.za/cropbiolife/ 08/09/16