FIELD PERFORMANCE AND BIOACTIVE CONTENT OF *IN-VITRO* DERIVED ALOE VERA (*Aloe vera* L. Burm. F.) AS INFLUENCED BY PLANTING DISTANCE, FERTILIZATION AND PLANT MATERIAL

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ABSTRACT

Four (4) sets of *Aloe vera* plantlets propagated *in-vitro* and acclimatized in four potting media – designated as planting material types (PM) – were cultivated in open field to evaluate growth, yield and bioactive content and subjected to three (3) planting distances (PD) and three (3) fertilizer types (F). The four potting media were PM1- in garden soil (GS), PM2 - in garden soil and sand medium (GS:S) 1:1 v/v, PM3 - in garden soil and carbonized rice hulls (GS:CRH) 1:1 v/v and PM4 - in garden soil and coco peat (GS:CP) 1:1 v/v. Fresh weight and volume of leaves of mother plant in the 10th (last) month were affected by PD. All planting material treatment combinations used except PM1, PM2, PM3 and PM4 significantly differed in harvested volume of peel. GS-CP (PM4) and GS-CRH (PM3) hardened propagules significantly increased the volume of gel against GS (PM1). At 60cm x 45cm, %N in leaves was highest but comparable %N at 60cm x 30cm while %P in leaves was significantly higher with chicken manure urea-solophos-muriate of potash. %P in leaves was higher in plants acclimatized in GS-CRH compared to plants acclimatized in GS-CP. ppm K in leaves at 60cm x 30cm spacing was significantly higher than at 60cm x 60cm. Aloin A in leaves was significantly higher with complete fertilizer than with chicken manure-urea-solophos-muriate of potash. Aloe emodin production of plants acclimatized in GS-S differed significantly between plants spaced at 60x45 cm and 60x30 cm, and between plants applied with complete fertilizer against chicken manure-urea-solophos-muriate of potash. Aloe emodin per unit area varied significantly between GS and GS-S at 60x30 cm.

Key words: Aloin, Aloe emodin, cocopeat, secondary metabolites, vermicast

INTRODUCTION

Aloe vera is a perennial plant that grows into the shape of a tuft with a base of spirally-arranged succulent, thorny-edged leaves (Bassetti and Sala 2005). Grown in warm tropical areas, it cannot survive freezing temperatures (Pandey and Singh 2016). *Aloe vera* is a source of valuable highly functional ingredients in the cosmetic, pharmaceutical and food industries (Ramachandra and Rao, 2008) so that it is cultivated as a major high-value commodity around the world (Das and Chattopadhay 2004). In 2004, the global turnover of fresh Aloe vera gel products was valued at approximately US$ 125 million and was expected to grow at a rate of 35% over the next five years (Afolayan, and Adebola 2006).

In foreign cultivation settings, volumes of *Aloe vera* planting materials are required when large scale production is to be undertaken and the use of *in vitro*-propagation for planting material is seen applicable (Cristiano 2016). This method of plant multiplication was long been proven effective (Natali et al. 1990) but field performance of these planting materials acclimatized in any particular potting medium has not yet been tested. However, the success with micropropagation for mass propagation depends upon the plants' survival after separation from the culture bottles (Hazarika 2003). Micropropagated plants are delicate and should be acclimatized first for them to develop durable traits that will help them survive in the field (Pospisilova et al. 1999).

Various international production guides for this plant suggest diverse plant spacings owing to differences in soil, terrain, elevation and climate, such as 60cm x 60cm and 45cm x 45cm row-hill spacing in several provinces of India (Cristiano 2016). In plant density study, sweet pepper increased total yield when planted at closer spacing but vegetative growth and production of reproductive structures decrease (Aminifard et al. 2012). In onion, wider planting distance has been found to increase the number of leaves per plant and weight of large size bulbs (Dawar et. al. 2007). In its natural habitat, *Aloe vera* grows without fertilizers although nitrogen application boosts yield (Saha et al. 2005). In cultivated Aloes, the application of 8-10 tons/ha of farmyard manure (FYM) before land preparation and the incorporation of 35-70-70 kg/ha N, P_2O_5 and K_2O before the last plowing is recommended (Manvitha and Bidya 2014).

In the Philippines, however, there is yet no published report that emphasized on a standard potting media for greenhouse acclimatization of tissue cultured *Aloe vera*. Likewise, an appropriate fertilization program and planting distance recommendation for field cultivation of *Aloe vera* from either *in-vitro* derived regenerants or even suckers derived from backyard grown mother plants have not been established in the Philippines. Nutrient management and maintenance of optimum number of plants per unit area are important crop management practices for enhanced crop productivity (Sumathi et al. 2013) such as in the field production of *Aloe vera*. Hence, this study pioneers the exploration of appropriate cultural management for local commercial production by evaluating planting distance and soil nutrient management. To our knowledge, this is the first paper to report locally on the effect of plant spacing and fertilization on growth and yield performance of *Aloe vera*, and their effects on levels of bioactive compounds (Aloin A and Aloe emodin).

MATERIALS AND METHODS

The study was conducted in June 2016 – September 2017 at the University of the Philippines Los Baños (UPLB), Laguna Philippines, specifically at the Vegetable Production Site of the Institute of Crop Science for the field experiment and at the Analytical Service Laboratory of the Institute of Plant Breeding for quantification of secondary metabolites. The experiment was laid out as a 3-factor experiment in split-split plot design with three replications. Planting distance (PD) with row-hill spacing of 60cm x 60cm (PD1), 60cm x 45cm (PD2) and 60 cm x 30cm (PD3) was assigned as the main plot. Fertilizer type (F) was the sub-plot while planting material type (PM) was assigned to the sub-subplot.

A total of 2,376 of *in vitro*-derived plantlets acclimatized in four (4) different potting media were designated as planting material types (PM): PM1:Garden soil (GS), PM2: Garden soil-Sand (GS-S) 1:1 v/v, PM3: Garden soil-Carbonized rice hull (GS-CRH) 1:1 v/v and PM4: Garden soil-Cocopeat (GS-CP) 1:1 v/v. Volume of each potting media during acclimatization was made equal to 52 cm^3. Fertilizer was applied at the recommended rate of 50-50-50 NPK kg/hectare (Owoade and Adeoye, 2016) using the fertilizer types in Table 1. Vermicast and chicken manure were incorporated into the soil in each planting hole at three days before transplanting. Urea, solophos and muriate of potash were applied in three (3) split applications at 108 days interval.

Cultural management. An area of 1,081.6 m^2 (34.5 m x 31.35 m) was ploughed and harrowed twice to a depth of 20 cm to improve aeration and soil homogeneity. The area was divided into three (3) plots for the three (3) planting distances, maintaining borders 1 meter wide between replications and main plots, and 0.75-meter-wide between sub plots and sub-subplots within replications. Prior to field transplanting, weight of all plantlets chosen as samples were maintained at or closer to 4 grams each. The field temperature, relative humidity, soil temperature, sunshine duration and soil moisture during transplanting was 27.43^{o}C, 87.71%, 31.57^{o}C, 2.76 hours and 6.86% respectively. Acclimatized plantlets were transplanted to the field at 15, 21 and 30 plantlets for PD1, PD2 and PD3, respectively (Fig. 1A). Watering was done immediately after transplanting and repeated during dry days. Hilling up was done after each weeding or fertilizer application. Ten (10) months after transplanting, harvesting was done by pulling off the three (3) lower-most leaves (Fig.1B).

Fig. 1. *Aloe vera* in the 5th (A) month of field cultivation and harvested leaf samples at 10 months after cultivation (B)

Table 1. Types of fertilizer materials and their rates application per *Aloe vera* plant across three (3) planting distances

				PD1	PD2	PD3
Planting distance (cm)				60 x 60	60 x 45	60 x 30
Plant density (plants/ha)				27,777	37,037	55,555
No. of plants per treatment				540	756	1,080
Treatment	**Fertilizer material**	**NPK Composition**	**Actual Amount* (kg/ha)**	**Rate per plant (g)**	**Rate per plant (g)**	**Rate per plant (g)**
F1	CF	14 – 14 – 14	357†	12.85†	9.64†	6.43†
F2	Vermicast	0.59-0.5-0.79	3,164.56	113.93	85.44	56.96
	Urea	46-0-0	68.11†	2.45†	1.83†	1.26†
	Solophos	0-20-0	170.85†	6.15†	4.61†	3.80†
F3	CHM	4.23 -2.42-3.03	591.02	21.27	15.95	10.64
	Urea	46-0-0	54.35†	1.96†	1.47†	0.97†
	Solophos	0-20-0	178.5†	6.42†	4.81†	3.21†
	MP	0-0-60_	53.50†	1.93†	1.45†	0.96†

Complete Fertilizer (CF); Urea (U); Vermicast (VC); Chicken Manure (CHM); Muriate of Potash (MP)
* Rate of application (kg/ha) that would meet the 50-50-50 kg/ha NPK,
† Split application of three (3) equal parts

Growth, biomass and yield parameter. Data on growth parameters were collected bi-monthly. Leaves and suckers from each sample mother plant were counted, excluding apical shoots shorter than one (1) cm and dried leaves. The volume of leaves was computed as $V = (L|12)\Pi wt$ (Hernandez-Cruz et al. 2002), where V= Volume of leaves (cm^3/plant); L = Length of leaf (cm); W = Width of leaf (cm); T = Thickness of leaf (cm); π=pi (3.14); 12 = correction factor. Leaf length, width and thickness were measured bimonthly. Length was measured from the leaf base to the apex, while thickness and width were measured at the midpoint of the leaf length.

After washing off soil from roots, sample plants were air-dried for 20 min before fresh weight was taken. To obtain plant dry weight, plants were oven-dried at 70°C for 24 hrs or until brittle. Fresh and dry weights of suckers collected from each mother plant were obtained using the same procedure. The sum of the fresh/dry weights of mother plant and its suckers were recorded as total fresh/dry weight of mother plant with suckers.

To determine Mean Crop Growth Rate (MCGR), bimonthly crop growth rate was computed as:

$$CGR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{1}{GA}$$

Where: CGR = crop growth rate (g m^{-2} mo^{-1}); W_1 = initial dry weight of mother plant (g); W_2 = final dry weight of mother plant (g); t_1 = time at the start of sampling period; t_2 = time at the end of sampling period; GA = ground area (m^2). MCGR is then taken as the average of the computed bimonthly CGRs. Time of leaf whorling was the number of weeks to complete phyllotaxis.

For peel and gel yield, the weight and volume of three (3) lower-most leaves harvested from each mother plant were obtained. Harvested leaves were weighed on a digital scale while volume was computed using the formula by Hernandez-Cruz et al. (2002). Then, leaves were peeled to obtain peel and gel weights separately. The volume of harvested peel (V_p) and gel (V_g) were estimated using the following formulae:

$$V_p = \frac{V_L}{W_L} \times W_p \qquad V_g = \frac{V_L}{W_L} \text{ x } W_g$$

where: V_p = volume of harvested peel (cm^3); V_g = volume of harvested gel (cm^3); V_L = volume of harvested leaves (cm^3); W_L = weight of harvested leaves (g); W_p = weight of harvested peel (g).

Quantification of macroelement contents in leaves. Three (3) mature (lower-most) leaves were collected from the remaining plants in replications 1 and 2 of each treatment and weighed. The leaves were oven dried at 60ºC for 24 hours until brittle. The dried leaf samples were weighed and then pulverized with a motorized grinding machine. From each sample plant, five (5) grams of pulverized leaves were submitted to the Analytical Services Laboratory of the Agricultural Systems Institute, UPLB for analysis of N, P and K contents using Kjeldahl method, spectrophotometric method and flame photometric method, respectively.

Measurement of bioactive substances. Detection and measurement of Aloin A and Aloe emodin present in the aloe extracts were done with the use of reversed-phase high performance liquid chromatography (RP-HPLC) with UV-Vis detector. Following the protocol described by Tan et al. (2011), extracts for analysis were prepared from three (3) whole leaf samples taken from randomly selected plants from the middle of planting rows in each treatment/replication.

Statistical analysis. Analysis of Variance (ANOVA) was conducted to test for significant differences in means of the parameters across treatments. Pairwise comparison of means was done using Tukey's HSD, Dunn's or Scheffe tests where appropriate. Tests were done at 5% level of significance.

RESULTS AND DISCUSSION

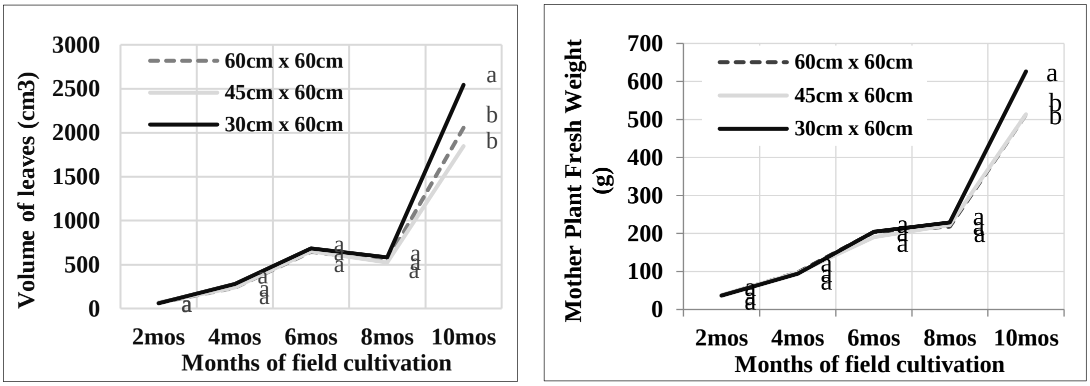
Effect of planting distance on volume of leaves (g) and fresh weight (g) of mother plants. In the 10th month of field cultivation, 60cm x 30cm spacing resulted in mean leaf volume and fresh weight of mother plant significantly higher than those obtained from PDs of 60cm x 60cm and 60cm x 45 cm by 37.7% and 23.7%, respectively (Table 2). Leaf volumes (Fig. 2A) and fresh weights (Fig. 2B) at the wider distances, PD1 and PD2, were comparable to each other. Closer plant spacing improved accumulation of fresh biomass as indicated by significantly greater volume and fresh weight of leaves of mother plants at 60cm x 30cm compared to the wider PDs. Horizontal leaf orientation when plants are farther apart can expose the plants to high irradiances that can cause photoinhibition and eventual cessation of growth. Closer together, leaves tend towards a more vertical orientation as a result of crowding. Hence, they are not directly exposed to solar radiation. The shading of the lower leaves and the soil beneath reduces transpiration and soil moisture loss. The moisture-saving effect of closer spacing significantly promoted the growth of *Aloe vera* plants. Although interplant competition at closer spacing is higher, a less dense plant population at wider spacing may also seriously inhibit uptake of nitrogen as a result of rapid evaporation upon frequent exposure of soil surface to intense irradiance. Also, more weeds grow in wider spacing which directly competes for nutrients. These findings were consistent with the observation made by Islam et al. (2017) that plant spacing and fertilizer application have significant influence on crop growth and yield. High agricultural yields depend strongly on fertilization with mineral nutrients, while plant density influences canopy architecture, light conversion efficiency, duration of vegetative growth, dry matter production and ultimately, the economic productivity of a crop (Al-Suhaibani et al. 2013).

Table 2. Summary of analyses of variance on the effects of PD, F and PM on growth and yield parameters of *Aloe vera* on the 10th month of field cultivation.

Growth and Yield Parameters	Sources of Variation						
	PD	F	PM	PDxF	PDxPM	FxPM	PDxFxPM
Fresh weight of mother plant (g)	*	ns	ns	ns	ns	ns	ns
Number of leaves	ns	ns	ns	ns	ns	ns	ns
Volume of leaves (cm^3/plant)	*	ns	ns	ns	ns	ns	ns
Number of suckers	ns	ns	ns	ns	ns	ns	ns
Crop growth rate ($g.dm^{-2}$ $60d^{-1)}$	ns	ns	ns	ns	ns	ns	ns
Time to whorling (weeks)	ns	ns	ns	ns	ns	ns	ns
Weight of harvested leaves (g/plant)	ns	ns	ns	ns	ns	ns	ns
Volume of harvested leaves (cm^3/plant)	ns	ns	ns	ns	ns	ns	ns
Weight of harvested peel (g/plant)	ns	ns	ns	ns	ns	ns	ns
Volume of harvested peel (cm^3/plant)	ns	ns	*	ns	ns	ns	ns
Weight of harvested gel (g/plant)	ns	ns	ns	ns	ns	ns	ns
Volume of harvested gel (cm^3/plant)	ns	ns	*	ns	ns	ns	ns

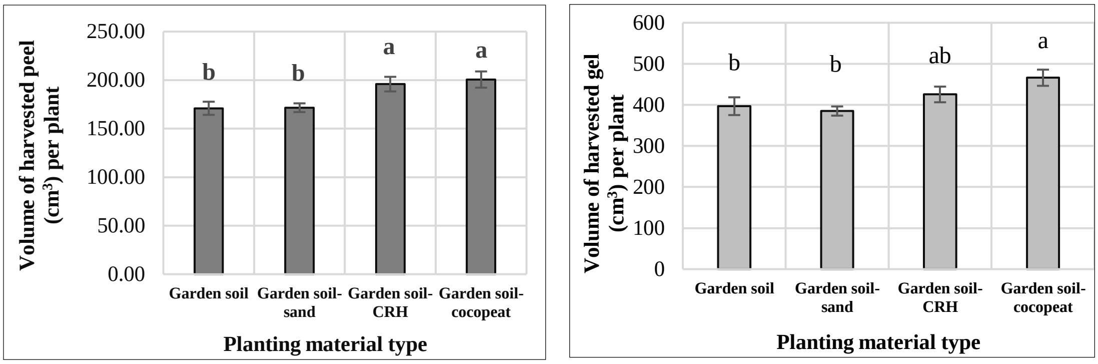
* = with means significantly different; ns = with means not significantly different.

Effect of type of planting material on volume of harvested peel and gel. Planting materials acclimatized in GS-CP produced the highest volumes of peel and gel although these were statistically comparable to those obtained from planting materials acclimatized in GS-CRH. Peel volume (Fig.3A) and gel (Fig.3B) produced with GS-CP were significantly higher than those of plant materials acclimatized in GS-S and GS by 14.86% and 17.39%, respectively. These findings also revealed the beneficial effects of organic matter as a garden soil enhancer for potting media during acclimatization on volumes of harvested peel and gel, particularly CP and CRH. The yield of Aloe gel was better with low frequency of watering and high amount of fertilizers (Hernandez-Cruz et al. 2002). The incorporation of CP-based potting medium into the field at transplanting may have provided organic materials that regulated soil moisture (Bot and Benitez 2005), while relatively higher nutrient content of GS-CP mixture became available for use by plants during the growing period. Soil moisture in closely planted crops remains available because water evaporation is inhibited by shading of canopies (Qui et al. 2012). Crowding of plant leaves kept soil moisture conserved by preventing irradiance to penetrate into the soil surface and dry it up. Thus, closer spacing resulted in better growth – heavier mother plants and larger leaves.



(A) (B)

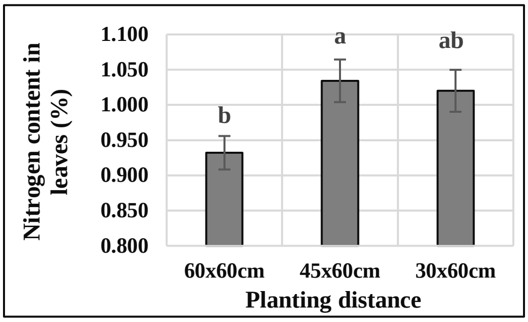
Fig. 2. Bi-monthly volume of leaves (A) and fresh weight (B) of *Aloe vera* mother plants as affected by PD in the 10^{th} month of field cultivation. Points with a common letter at each sampling period are not significantly different at 5% level.



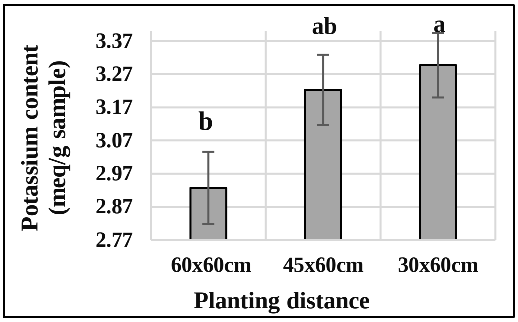
(A) (B)

Fig. 3. Volumes of harvested peel (A) and gel (B) of *Aloe vera* as affected by PM in the 10^{th} month of field cultivation. Bars with a common letter are not significantly different at 5% level. Standard errors are represented by vertical line bars.

Effect of treatments on macroelements content in leaves. Nitrogen and potassium content in leaves of *Aloe vera* plants were favored by closer plant spacing. Nitrogen content of plants at 60cm x 45cm was significantly higher than %N in leaves at 60cm x 60cm spacing, but statistically comparable to %N in leaves at 60cm x 30cm (Fig. 4A). Potassium content in leaves, on the other hand, was significantly higher at 60cm x 30cm, comparable to %K in leaves at 60cm x 45cm (Fig. 4B).The availability of nitrogen also translated into significantly higher %N in leaves at 60cm x 45cm which was comparable to %N at 60cm x 30cm further confirms the advantage gained by moisture saving as discussed above. Phosphorus content in leaves, on the other hand, was favored by the incorporation of CRH into the potting mix during acclimatization. Biochar (CRH) has a relatively high phosphorus content and when applied to the soil, also has the effect of making available phosphorus fixed in the soil by increasing soil pH (Zheng et al. 2013). As the GS-CRH potting medium was incorporated into the field during transplanting, phosphorus became more readily available to plants, translating into higher %P in leaves. Also, there was significant effect of FxPD on phosphorus content in leaves. At wider planting distances (PD1 and PD2), a significant increase in leaf phosphorus was observed over closer spacing when chicken manure-urea-solophos-muriate of potash (F3) was used. Inter-plant competition at closer spacing may have hampered uptake of this mineral. The organic feature of chicken manure in the fertilizer mix may have aided the absorption of minerals more than inorganic fertilizers (Courtney and Mullen 2007), particularly over the duration of field cultivation.



(A)



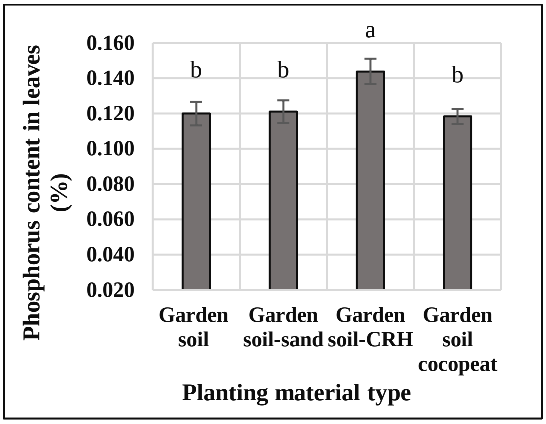
(B)

Fig. 4. Mean nitrogen (A) and potassium (B) contents in leaves of field cultivated *Aloe vera* plant as affected by PD. Bars with the same letter are not significantly different at 5% level. Standard errors are represented by vertical line bars.

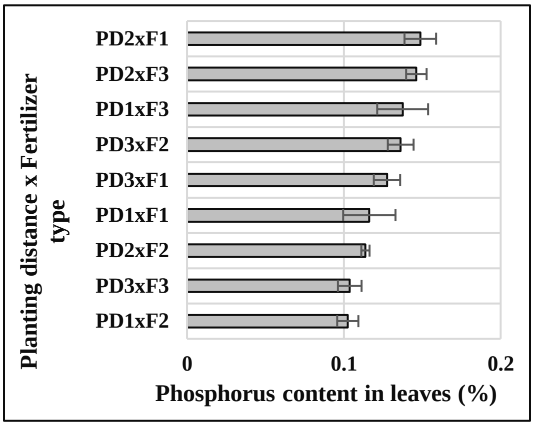
Potassium content in *A. vera* leaves was favored by closer plant spacing of 60cm x 30cm due to availability of soil moisture conserved at longer period upon shading of crowding canopies. Soil moisture improves the solubility and availability of nutrients in the soil (Roy et al. 2006), acting as the medium for potassium ion movement to the root surface (Taiz and Zeiger 2002). Furthermore, potassium uptake in wider spaced plants may have been limited by weed competition as observed in the field. In drier soils, potassium uptake is inhibited by lack of solvent to carry potassium ions into the rhizosphere. Roy et al. (2006) stressed that the availability of mineral potassium (K+) and other cations is improved by a satisfactory soil moisture status.

Phosphorus content in leaves of field planted *Aloe vera* was significantly higher with plants acclimatized in GS-CRH than in GS-CP, GS-S or in GS (Fig. 5A). However, the interaction between planting distance and fertilizer type resulted in significantly highest % P in leaves at 60cm x 45cm with the application of 50-50-50 kg/ha NPK using complete fertilizer or PD2xF1 (Fig. 5B), comparable to P content in leaves of plants applied with chicken-manure-urea-solophos-muriate-of-potash combination at the same plant spacing (PD2xF3). Concomitantly, on the 10th month of cultivation as shown in Table 4, PD had significant effect on fresh weight of mother plants and volume of leaves, while PM showed significant effect on harvested peel and gel. Fertilizer type and treatment interactions did not have significant effects. Moreover, treatments and their interactions did not show significant effects on growth and yield parameters in the first nine (9) months of cultivation. Misra et al. (2000) stressed that wider plant spaces reduce interplant competition for nutrients, resulting in better nutrient uptake by plants. A widely-spaced plant stand provides more space for root elongation and a bigger area for sourcing of phosphorus by the plant. This is in conformity with observations made on the effect of wide spacing on P uptake by broccoli (Roni et al. 2014), black nightshade (Sivakumar and Ponnusami 2011) and hybrid maize (Srikanth et. al. 2009). However, high concentration of P in the soil is not always proportional to the accumulation of phosphorus in the leaves of plants. For instance, Gautam et al. (2012) reported that at high population density, corn plants had low phosphorus content even in soil with high P concentration. On the contrary, Ravichandran and Srinivasan (2017) noted a significantly higher phosphorus uptake of hybrid sunflower at 30cm x 30cm spacing compared to 60cm x 30cm. In the present study, higher phosphorus content in A. vera leaves coincided with nitrogen concentration at 60cm x 45cm spacing. The high P content may be associated with high N content. Several studies revealed that phosphorus content in leaves increases with an increase in nitrogen uptake at the same plant spacing (Ravichandran and Srinivasan 2017; Sivakumar and Ponnusami 2011; Srikanth et al. 2009). Increase in P content of *A. vera* under chicken manure-urea-solophos-muriate of potash fertilizer combination may be attributed to chicken manure as an organic fertilizer.

The use of organic fertilizers could increase phosphorus content in leaves of *Aloe vera* (Saha et al. 2005). Organic matter is a good source of plant-available nutrients and incorporation of this material into soil could maintain high microbial populations and activities (Arancon and Edwards 2005). High microbial population increases the probability of mineralization necessary for nutrients to become available to plants. Studies revealed that phosphorus derived from organic sources is more effective for plant absorption than inorganic origin (Courtney and Mullen 2007; Gil et al. 2007). This is because mineralization and assimilation of this nutrient are microorganism mediated processes (Adhami et al. 2014.).



(A)



(B)

Fig. 5. Mean phosphorus content in leaves of field cultivated *Aloe vera* plants as affected by PM (A) and by PDxF (B). Bars with the same letter are not significantly different at 5% level. Standard errors are represented by vertical line bars.

Treatments effect on secondary metabolites content.

Aloin A. The concentration of Aloin A in leaves of plants applied with complete fertilizer was significantly higher by 25.56% (403.180 µg/g dry leaf powder) than with chicken-manure-urea-solophos-muriate-of-potash (300.010 µg/g dry leaf powder). However, this was comparable to that of plants applied with vermicast-chicken manure fertilizer combination (340.405µg/g leaf powder) (Fig. 6; Table 5). Aloin is the major anthraquinone component of Aloe vera. It is composed of a mixture of two diastereo-isomers, Aloin A and Aloin B (Afolayan and Adebola 2006). Dried exudate contains 15–40% anthrone 10-C-glucosides which has numerous actions, i.e., laxative, blood purifying, and diuretic (Bassetti and Sala 2005). Aloe emodin, on the other hand, is an anthraquinone molecule present in the yellow exuded matter found in the lining under the cuticle of the Aloe leaf (Bassetti and Sala 2005). It is present in Aloe latex in small amounts relative to Aloin A and Aloin B, can be formed by oxidative cleavage of the glycosidic linkage of Aloin A or Aloin B (Wang et al. 2012). Thus, the production of Aloe emodin was considered dependent on the accumulation of Aloin A and

B in *A. vera* plants. Aloin A was enhanced by the application of inorganic fertilizers while Aloe emodin was influenced by treatment interactions. The effect of complete fertilizer in increasing Aloin A level could be due to the immediate availability of nutrients in the soil, unlike the slow release of nutrients from organic fertilizers like vermicast and chicken manure. However, the extent of mineralization and nutrient availability in the soil from organic fertilizers is still undetermined. In a study on *Aloe vera* fertilization, inorganic fertilizer at the rate of 40-40-40 and 80-80-80 kg/ha NPK led to a significant increase in Aloin content in leaves (Saradhi et al. 2007).

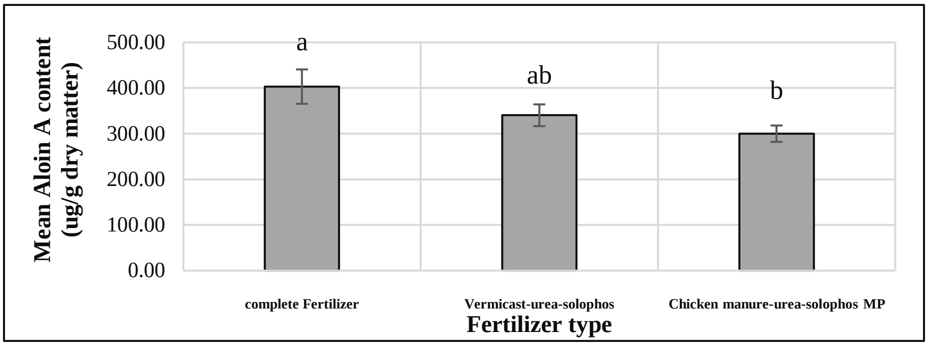


Fig. 6. Mean Aloin A concentrations (ug/g dry matter) in harvested leaves of field cultivated *Aloe vera* plants as affected by F. Bars with a common letter are not significantly different at 5% level. Standard errors are represented by vertical line bars.

Aloe emodin yield per plant. Aloe emodin content per plant was significantly highest with plants at 60cm x 30cm spacing applied with complete fertilizer derived from plantlets acclimatized in GS-S (PD3xF1xPM2) (Fig. 7). In this study, highest Aloe emodin in each plant was produced at 60cm x 45cm where optimum growth factors (i.e. light, nutrients and moisture) were assumed to be received by the plants as compared to wider and closer planting hills. This is consistent with the results of the study by Nematian, et al. (2015) wherein Aloe emodin content of *Aloe vera* was significantly higher in medium plant density of 4 plants/m^2 (50x50 cm) compared to 2 plants/m^2 and 6 plants/m^2. The active substance biosynthesis of *Aloe vera* depends on light treatments and plant respiration (Alagukannan et al. 2008). Significant increase in crude Aloin was observed for plants grown under diffused sunlight compared to plants grown under direct sunlight with high irradiance (Tawfik et al. 2001). Therefore, in wider and closer spaced plants, too much and too little light led to lesser accumulation of Aloe emodin.

Table 3. Secondary metabolites content of *Aloe vera* as affected by PD, F and PM after 10th month of field cultivation.

Secondary metabolite content	Sources of variation						
	PD	F	PM	PDxF	PDxPM	FxPM	PDxFxPM
Aloin A contentration (µg/g dm)	ns	*	ns	ns	ns	ns	ns
Aloin A content (mg/per plant)	ns	ns	ns	ns	ns	ns	ns
Aloin A per unit area (mg/m2)	ns	ns	ns	ns	ns	ns	ns
Aloe emodin contentration (µg/g dm)	ns	ns	ns	ns	ns	ns	ns
Aloe emodin content (mg/per plant)	ns	ns	ns	ns	ns	ns	*
Aloe emodin per unit area (mg/m2)	ns	ns	ns	ns	*	ns	ns

* = with means significantly different; ns = with means not significantly different.

Aloe emodin yield per unit area. Significantly, highest Aloe emodin yield per unit area was obtained at 60cm x 30cm with plants acclimatized in GS-S (PD3xPM2), while lowest was at 60cm x

60cm spacing with the same PM (PD1xPM2) (Fig. 8). Lowest Aloe emodin yield per unit area was obtained with wide spacing of 60cm x 60cm, most expecially with sand-garden soil potting medium. Closest planting distance generally produced high Aloe emodin yield per unit area, owing perhaps to the higher plant density but not with plants acclimatized in pure garden soil medium. Aloe emodin content per unit area (mg/m-2) observed in pure garden soil (PM1) was significantly higher against sand-garden soil (PM2) potting media and may be due to greater concentration of the compound per dry matter of the leaves which was found to be higher (32.43 ug/g) in PM1 than in PM2 (30.01ug/g). This was only observed at 60cm x 30cm (PD3) spacing because of the conditions created by higher plant density that led to larger plants as manifested in higher fresh weight and volume of leaves. Yield was also greater with PM1 (0.307 mg/plant) than with PM2 (0.282mg/plant). Highest Aloe emodin produced in 60cm x 30cm spacing (PD3) of 1.66 mg/m2 was significantly higher than that obtained at 45cm x 60cm (PD2) and 60cm x 60cm (PD1) of 1.259 and 0.776 mg/m2, respectively. This was mainly due to higher number plants contained per unit area as well as the greater leaf volume produced per plant. The results were observed at with sand-garden soil (PM2) and CRH-garden soil (PM3) potting media. Thus, higher Aloe emodin will be produced when Aloe vera is planted at 60cm x 30cm spacing using micropropagated planting materials acclimatized with either sand-garden soil (PM2) or CRH-garden soil (PM3) potting media. Also, under cocopeat-garden soil (PM4) potting medium, plants grown at closest spacing produced significantly higher Aloe emodin than those in widest plant spacing which is primarily the result of higher plant density.

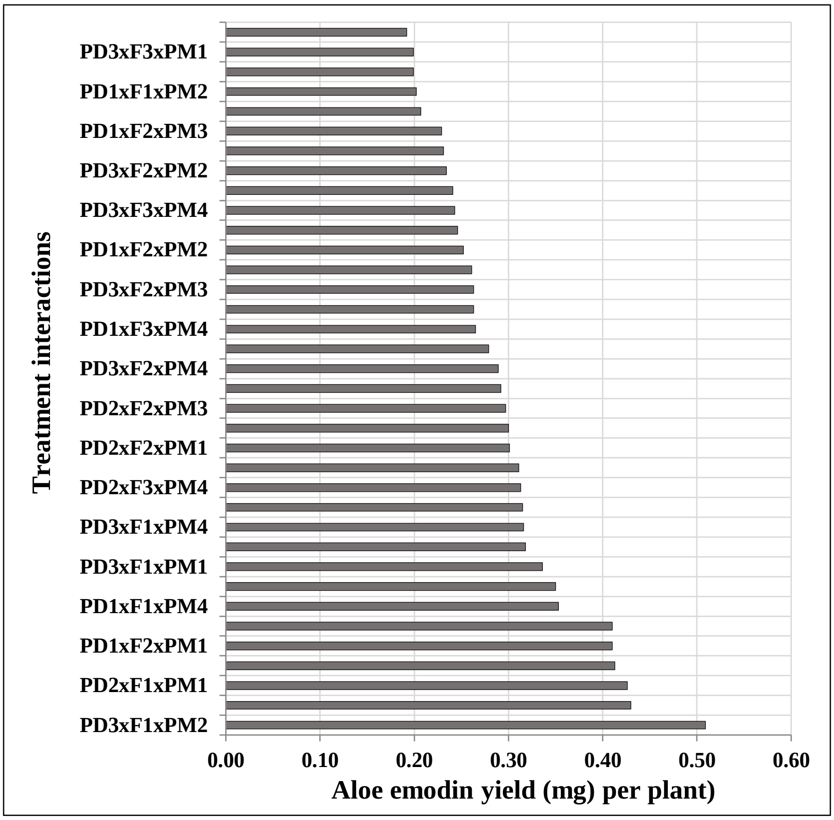


Fig. 7. Aloe emodin yield per plant (mg/plant) as affected by PDxFxPM

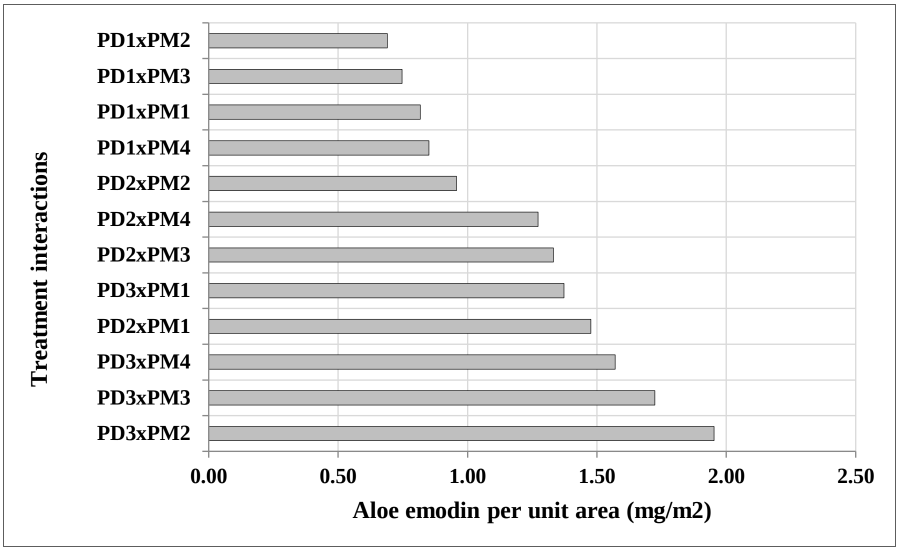


Fig. 8. Aloe emodin yield per unit area (mg/m2) as influenced by PDxPM.

CONCLUSION

There are specific and combined treatments identified in each of the significantly improved parameters. Biomass accumulation in *Aloe vera* plantlets propagated *in-vitro* was found responsive at closer planting distance and in cocopeat-based acclimatized planting materials (GS:CP) in peel and gel production. Nitrogen accumulation in leaves was effective in medium inter-row spacing while potassium content at closer planting distance. The use of planting materials hardened in garden soil-carbonized rice hull potting medium (GS:CRH) under complete fertilizer nourishment performs well in foliage phosphorus production. However, complete fertilizer improves the synthesis of secondary metabolite Aloin A whilst Aloe emodin per plant was enhanced in plantlets acclimatized under garden soil-sand, grown at closer plant spacing with complete fertilizer application.

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