EVALUATION OF SUBSTRATES ON THE EMERGENCE OF "ARATICUM-DE-TERRA-FRIA" (*Annona emarginata* (Schltdl.) H. Rainer) SEEDLINGS[1]

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ABSTRACT - Choosing a substrate is the determinant factor for the seedling producer; thus, the aim of this study was to evaluate the effect of different types of substrates on the emergence of "araticum-de-terra-fria" (*Annona emarginata* (Schltdl.) H. Rainer) seedlings. The experiment was carried out in a greenhouse and the experimental design was in randomized blocks, with three treatments and five replicates of 72 seeds per plot. The treatments consisted of the following substrates: coconut fiber, vermiculite and Plantmax® Citrus. The number of emerged seedlings was weekly counted for 105 days. Data regarding seedling height were obtained, and the emergence velocity index and mean time, besides total emergence percentage and that over time were calculated. Results from total mean emergence percentage, seedling height, emergence velocity index (EVI), and mean emergence time (MET) were subjected to analysis of variance and means were compared by the Tukey's test at 5% significance. The curves concerning the emergence percentage over time were fit by the logistic growth equation for each treatment and the means of each parameter (A, B, C) were compared by the Duncan's test at 5% significance. The substrates vermiculite led to the highest values of emergence percentage differing from the PlantMax® Citrus, but not of the coconut fiber, however the vermiculite promoted seedling height in a shorter time; therefore, this substrate is recommended for the initial development of "araticum-de-terra-fria" (*Annona emarginata* (Schltdl.) H. Rainer) seedlings.
Index terms: Annonaceae, seeds, seedlings, logistic growth equation, nursery[1].

AVALIAÇÃO DE SUBSTRATOS NA EMERGÊNCIA DE PLÂNTULAS DE ARATICUM-DE-TERRA-FRIA (*Annona emarginata* (Schltdl.) H. Rainer)

RESUMO - A escolha do substrato é fator determinante para o produtor de mudas e, portanto, o objetivo deste trabalho foi avaliar o efeito de diferentes tipos de substratos na emergência de plântulas de araticum-de-terra-fria (*Annona emarginata* (Schltdl.) H. Rainer). O experimento foi conduzido em casa de vegetação, em delineamento experimental de blocos ao acaso, com três tratamentos e cinco repetições de 72 sementes por parcela. Os tratamentos foram constituídos pelos substratos: fibra de coco; vermiculita e PlantMax® Cítrus. A contagem do número de plântulas emergidas foi realizada semanalmente durante 105 dias. Foram obtidos dados de altura de plântulas e calculados o índice de velocidade de emergência, o tempo médio e a porcentagem de emergência ao longo do tempo e total. Os dados de porcentagem de emergência média total, altura de plântulas, índice de velocidade de emergência (IVE) e tempo médio de emergência (TME) foram submetidos à análise de variância, e as médias, comparadas pelo teste de Tukey, a 5% de probabilidade. As curvas da porcentagem de emergência ao longo do tempo foram ajustadas pela equação logística de crescimento, para cada tratamento, e as médias de cada parâmetros (A,B,C), comparadas pelo teste de Duncan, a 5% de probabilidade. O substrato vermiculita foi o que proporcionou os maiores valores de porcentagem de emergência, diferindo do PlantMax® cítrus, mas não da fibra de coco, porém a vermiculita promoveu maior velocidade de emergência e maior altura de plântulas em menor tempo, sendo, portanto, recomendada para o desenvolvimento inicial de plântulas de araticum-de-terra-fria (*Annona emarginata* (Schltdl.) H. Rainer).
Termos de indexação: Annonaceae, sementes, plântulas, equação logística de crescimento, viveiro.

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INTRODUCTION

Among Annonaceae family, four species have great economic importance (MOSCA et al., 2006), sweetsop (*Annona squamosa* L.) and soursop (*Annona muricata* L.) in tropical regions, besides cherimoya (*Annona cherimola* Mill.) and atemoya (*Annona cherimola* Mill. x *Annona squamosa* L.) in subtropical areas (KAVATI, 1992; KAVATI, 1998). So, to propagate Annonaceae, and species *Annona emarginata* (Schltdl.) H. Rainer, synonymy of *Rollinia* sp. has been used as rootstock due to its rusticity and adaptation to dry and flooded soils in places located at 950m above sea level (TOKUNAGA, 2005; RAINER, 2007).

Furthermore, aimed at keeping the constant increase in production fields, technical procedures such as the choice of suitable rootstocks must be adopted to assure the quality and the high productivity of orchards, since the commercial Annonaceae species are susceptible to pathogens present in the soil, including *Phytophthora nicotineae*, *Pythium* sp. and *Rhizoctonia solani* (BONAVENTURE, 1999; MANICA et al., 2003).

The substrate choice is the crucial step in the production of either ungrafted or rootstock seedlings (BARBOSA et al., 2003). Information regarding substrate type is essential in the germinative process and seedling set (WAGNER JÚNIOR et al., 2006), since substrate may vary according to structure, pH, aeration, water retention capability and degree of contamination by pathogens (KONDURU et al., 1999; BOOMAN, 2000; GONÇALVES et al., 2000; LIMA et al., 2001; SILVA et al., 2001), and may also affect germination and seedling development due to its different ways of making water, light, temperature, nutrients, and O_2 available (BEWLEY; BLACK, 1994; CARVALHO; NAKAGAWA, 2000).

The term substrate is used for every solid material, differently from soil, and may be natural, synthetic (phenolic foam, rockwool), residual (manure, bagasse, cotton fibers), mineral (perlite and vermiculite) or organic (peat, decomposed bark, coconut fiber) (WAGNER JÚNIOR et al., 2008). Such material placed in a container in its pure form or blended allows the development of the root system, thus playing a supportive role for plants (ABAD; NOGUEIRA, 1998; MULLER et al., 2000); besides, such material is free from phytopathogenic microorganisms and presents suitable nutritional and physical characteristics (GHINI, 2004).

Some aspects should be considered during the substrate choice, including seed size, humidity and light requirement, and installation ease (BRASIL, 2009).

The substrate known as coconut fiber is a material from industries that process coconut fibrous mesocarp, which is mainly found in countries of tropical climate (MARTINEZ, 2002). This substrate has high capacity of aeration and retention of easily available water. These materials also present other positive points such as a medium-to-high CEC, low C/N ratio (since the material contains high levels of hemicellulose and lignin), and acid (MARTINEZ, 2002) or neutral-to-alkaline pH (BATAGLIA; FURLANI, 2004). Coconut fiber is considered a good substrate for plants, presenting excellent physical characteristics for good root development (VERDONCK, 1984).

The substrate named vermiculite is an expanded mineral presenting medium, thin and very thin granulometry, in addition to special features that make it an important element in the composition of fertilizers and soil formation for horticulture and fruit culture, especially in dry periods (UGARTE et al., 2005). Since it is very light (80 to 140 kg/m^3 in the expanded form), this substrate is highlighted for facilitating the handling with composites containing soil, peat, pine bark, fertilizers, pesticides, and herbicides, besides promoting soil aeration while retaining moisture; it also has good cation exchange capacity, thus making ammonia, potassium and calcium available for the plants (POTTER, 2001). Another very important characteristic of vermiculite in seedling establishment is the natural sterility of this substrate, i.e. it is free from pathogens (FLORIANO, 2004).

The commercial substrate Plantmax® Citrus is produced from pine bark and presents low granulometry (GIRARDI et al., 2007). It contains the macro and micronutrients necessary for the initial development of seedlings (WAGNER JUNIOR et al. 2008). Since pine bark is included in its composition, Plantmax® Citrus presents good drainage, low water uptake capacity, acid pH, medium nutrient levels (95 meq/100g), and 20 to 40% of its particles smaller than 8 mm (MARTINEZ, 2002; BATAGLIA; FURLANI, 2004).

Thus, considering the importance of substrates for seedling formation, the present study aimed to evaluate different substrate types on the emergence of "araticum-de-terra-fria" (*Annona emarginata* (Schltdl.) H. Rainer) seedlings in order to help the procedures in nurseries.

MATERIAL AND METHODS

The experiment with substrates on the "Araticum-de-terra-fria" (*Annona emarginata* (Schltdl.) H. Rainer) emergence was carried out in a greenhouse, covered with a 150µm-thick transparent plastic film of polyethylene (anti-UV), from February to June 2008 in Botucatu Municipality, State of São Paulo, Brazil. The geographic coordinates of this locality are 48º 24' 35"W and 22º 49'10"S, and 800m mean altitude. According to the Köppen classification system, the climate in this region is Cfb transition to Cwb, relatively dry in the winter and rainy in the summer (TUBELIS et al., 1999).

Seeds were extracted in February 2008 and received phytosanitary treatment included sodium hypochlorite (1%) and the fungicide {(3a*R*,7a*S*)-2-[(trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione} (CAPTAN®) at 0.2% active ingredient.

After the phytosanitary management, the experimental design was in randomized blocks, with three treatments and five replicates of 72 seeds per plot.

Treatments consisted of the following substrates: coconut fiber; vermiculite and the commercial substrate PlantMax® Citrus. The seeds were sowed in trays of expanded polystyrene 67,0 cm of length per 34,0 cm of width, containing 72 emptied cells in the pyramidal form with 12 cm of height, 4,8 cm of side and 0,9 cm of opening in the inferior part of each cell in which the different substrates were distributed.

The evaluated variables were: **Total mean** emergence percentage and over time: represented by the percentage of emerged seedlings in the different substrate types at every 7 days and at the end of the experiment, 105 days after sowing (DAS); a seedling was considered emerged when its cotyledon was elevated above the substrate level; emergence velocity index (EVI): according to the method recommended by MAGUIRE (1962); the correction factor was suggested by SILVA AND NAKAGAWA (1995); mean emergence time (MET): calculated according to SILVA AND NAKAGAWA (1995), based on the number of emerged plants on each evaluation; **seedling** height: corresponded to the distance from the crown to the apex.

The curves regarding to the emergence percentage over time were fit through logistic growth equation for each treatment. Logistic functions were adjusted by using the SAS software (SAS INSTITUTE INC., 2002) and is represented by the equation $y = A / [1 + \exp(-(B+Cx))]$, where A is the parameter named asymptote, i.e. A is the growth limit of y when x grows indefinitely; the parameter B relates the initial growth (y for x=0) to the growth needed to reach the asymptote; and the parameter C is related to the derivative value in the curve inflection point for the same A value; the higher parameter C, the faster the growth reaches this point (CARVALHO, 1996).

Results of total mean emergence percentage, emergence velocity index, emergence mean time, and seedling height, besides those of the estimated parameters, were subjected to the Levene's test for homogeneity of variance. When the statistical prerequisites were met, results were subjected to analysis of variance. As regards results of total mean emergence percentage, emergence velocity index, emergence mean time, and seedling height, the Tukey's test was applied at 5% significance, whereas the Duncan's test was applied at 5% significance to the estimated parameters of the logistic growth equation.

RESULTS

The first "araticum-de-terra-fria" (*Annona emarginata* (Schltdl.) H. Rainer) seedlings were observed at 35 DAS (Figure 1), and the substrate vermiculite led to the highest emergence velocity, which was also observed in the results from the analysis of variance regarding to the estimated parameters of the logistic growth equation (Table 1). Verify with the vermiculite the biggest values for the parameter A (asymptote) in relation to substrate PlantMax® Citrus, besides both substrates did not differ of the coconut fiber substrate (Table 1).

However in the end of the experiment (105 DAS) were observed differences between the emergence velocity index (E.V.I.), the emergence mean time (M.E.T), and the seedling height in the substrate vermiculite in comparison with substrates coconut fiber and PlantMax®Citrus (Table 2). The biggest values of emergence percentage obtained at the end of the experiment (105 DAS) was of 87.5% (Figure 1 and Table 2) in the substrate vermiculite, demonstrating the same response pattern, which was significantly different from that detected by using Plantmax® Citrus (68.9%) (Table 2).

However, emergence was not synchronized (Figure 2), in others words, the emergence was distributed desuniform along the time what resulted in polymodal distribution. In this way a polymodal distribution of the relative frequencies regarding to seedling emergence was observed in all substrates (Figure 2), which indicates that the studied species does not present uniform emergence. However, vermiculite displacement curve to the left was detected, indicating that the emergence percentage was more concentrated at the beginning than at the end of the studied process.

The highest seedlings (7.38 cm) were observed by using vermiculite, followed by coconut fiber (6.76 cm) and Plantmax® Citrus (5.52 cm) (Table 2).

DISCUSSION

For seedling emergence it can be observed that is not necessary that germination process occurs properly. From the moment that germination is satisfactory, there is a greater chance that this seedling is established on a substrate and therefore the emergency occurs. Although the species is a little domesticated and has been reported to present dormancy (FINKELSTEIN et al., 2008), its metabolism was activated, resulting in high emergency percentage and subsequent seedling emergence.

In the present experiment, however, high emergence percentages were obtained only using different substrates, however, several studies with seeds of Annonaceae suggest that this family has rudimentary embryo, in other words, this embryo does not have a fully structured morphology, since its tissues are composed of little differentiated or undifferentiated cells (BEWLEY; BLACK, 1994; BEWLEY, 1997; MARCOS FILHO, 2005). This fact can then be one of the causes that lead to direct negative effects on germination and consequently the emergence (RIZZINI, 1973; BASKIN; BASKIN 1998; CAVALVANTE et al., 2007).

Therefore, it must be emphasized that high emergence percentages were observed by using different substrates, without application of plant growth regulators. Since the beginning of the experiment, vermiculite was more suitable to accelerate the emergence velocity (Figure 1 and Tables 1, 2) and to provide the highest values of total emergence percentage (87.5% though it has not differed from the substrate coconut fiber**)** and seedling height, suggesting its use during the sowing stage in nursery. The vermiculite was satisfactory for the germination and emergence of seeds from several species, such as cedar (*Cedrela odorata* L.) (ANDRADE et al., 1994), heart of palm (*Euterpe edulis* Mart.) (ANDRADE et al., 1999), "jenipapo" (*Genipa americana* L.) (ANDRADE et al., 2000), jequitibá-rosa (*Cariniana legalis* (Mart.), Brazilian rosewood (*Dalbergia nigra* (Vell.)) (RÊGO; POSSAMAI, 2002), "ipê-felpudo" (*Zeyhera tuberculosa* (Vell.) Bur.) (RAMOS et al., 2003), jatobá (*Hymenaea intermedia* Ducke var. *adenotricha* (Ducke) Lee & Lang.) (MELO et al., 2004), "itaubarana" (*Acosmium nitens* (Vog.) Yakovlev) (VARELA et al., 2005); "embiruçu" (*Pseudbombax grandiflorum* (Cav.) A. Robyns) (LOPES et al., 2007), mulungu (*Erythrina velutina* Willd.) (ALVES et al., 2008) and "angico-de-bezerro" (*Piptadenia moniliformis*) (PELACANI et al., 2009).

An explanation for such results is that the fundamental factors for seed germination and consequently the emergence is hydration (McLAREN; McDONALD, 2003; NOGUEIRA et al., 2003), since the germination process comprises a programmed series of hydrolysis reactions and new tissues, in addition to other factors (BEWLEY; BLACK, 1994; HILHORST, 1995; MARCOS FILHO, 2005).

Since seeds do not exhibit any physical barrier to water uptake, the higher germination substrate moisture seems to be crucial for triggering the process of seed germination and emergence (RESENDE, 2005). The good water retention capability of vermiculite (ZANETTI et al., 2003) in addition to the inherent characteristics that regulate water flow to seeds (VARELA et al., 2005) may have influenced the results (ALVINO; RAYOL, 2007). An adequate water supply leads to tissue rehydration, intensifying the respiration and reestablishing the metabolism, which culminates in visible germination and emergence of seedlings (CARVALHO; NAKAGAWA et al., 2000).

Thus, although vermiculite is not prescribed in the seed analysis rules (BRASIL, 2009), it has been recommended as an excellent substrate for seeds of large dimensions and varied shapes (FIGLIOLIA et al., 1993), since its low density (0.12 g/cm^3), high water retention capability (73.45%) and high aeration availability (FRETZ et al., 1979) led to greater contact of seeds with the substrate, together with high water supply and atmospheric oxygen to seeds (HARTMANN et al., 2002), which allowed the highest emergence velocity in this substrate. Larger seeds are potentially more vigorous, presenting a higher probability of successful seedling establishment, faster emergence (MARCOS FILHO et al., 1986; HAIG; WESTOBY, 1991; MENEZES et al., 1991; CARVALHO; NAKAGAWA, 2000) and well-developed root system; thus, substrates that keep humidity around seeds are necessary (FIGLIOLIA et al. 1993), which was observed by using vermiculite.

According to Carvalho and Nakagawa (2000), temperature influences the total emergency, besides its speed and uniformity, the water uptake speed, and consequently the biochemical reactions that determine the entire process. In addition, the probable association between light and temperature on seed germination and consequently the seedlings process must be considered (TAKAGI, 2004).

Temperature has a decisive influence on both germination and emergence process (BRANDEL, 2004). Responses to germination, seedling survival and growth according to the light intensity vary among plants from tropical dry forest (KHURANA; SINGH, 2001); however, these differences based on light intensity may not be observed under favorable environmental conditions (McLAREN; McDONALD, 2003; KERBAUY, 2008).

In the present study, although there was no temperature control inside the greenhouse, seeds remained on the substrates under the natural thermal oscillations of the environment in order to simulate the natural conditions under which the species is originally dispersed; this may have favored the emergence. The effect of alternate temperature on Annonaceae seed germination and emergence was evaluated by Ferreira et al. (2002), who studied atemoya (*Annona cherimola* Mill. x *Annona squamosa* L.) seeds and GA_3, and observed that 20-30°C alternate temperature was most suitable. As regards to the species "araticum-de-terra-fria" (*Rollinia* sp. synonymy of *Annona emarginata* (Schltdl.) H. Rainer) Rainer (2007), Costa and Ferreira (2008) reported that there was higher germination and emergence percentage in shorter time when seeds were subjected to 20/30ºC and 30/20°C alternate temperatures under any luminosity coSince vermiculite presents a buffering action capable of keeping the environment with higher moisture and aeration (PELACANI et al., 2009), besides making the ions potassium (K^+) and magnesium (Mg^{+2}) available (FRETZ et al., 1979), the stand may be formed in a shorter time, which is extremely important from an agronomic point of view (RIBEIRO et al., 2000). According to Nonogaki et al. (2010), the role of potassium on germination has not been well explained so far; however, this ion is known to be involved in water uptake for embryonic development. Petruzzelli (1988) stated that seed germination and consequently of emergence, besides being a structural component of the chlorophyll molecule, magnesium acts as an activator or regulator of several enzymes including ATPases, RuBP carboxylase/oxygenase and other enzymes from the carbohydrate metabolism (MARSCHNER, 1995), which in turn acts as energy source, keeping the metabolic processes; it also acts as source of material for structuring plant tissues, which will ensure seedling formation (BUCKERIDGE et al., 2004; KERBAUY, 2008).

High percentages of emergence, speed, and mean-time reduction are desirable requirements for seedling formation (PACHECO et al., 2006, MARTINS et al., 2009), and these characteristics were found in the present study; however, the emergence frequency of "araticum-de-terra-fria" seedlings was proven to be irregular, similarly to other species of the same genus, ranging from 10 days for *Annona squamosa* L. seeds (CORDEIRO et al., 2000), 25 to 30 days for *Annona muricata* L. (DONADIO et al., 1998), 60 days for atemoya (*Annona squamosa* L. x *Annona cherimola* Mill.) (TOKUNAGA, 2005), which was similar to the 61.3 days obtained in the present study, and 68.54 days for *Annona crassiflora* Mart. (CAVALCANTE et al. 2007). Thus, even the suitable substrate did not lead to a synchronized emergence, which corroborates the characteristic reported by Rizzini, (1973) and Lorenzi (2002) regarding non-uniform germination and emergence of Annonaceae species (SMET et al., 1999; BERNARDES et al., 2007; COSTA, 2009).

TABLE 1 - Means of the estimated parameters A, B and C from the logistic function compared by the Duncan's test.

Treatments/estimated parameters	A	B	C
T1 = Coconut fiber	77,3743 ab	7,0469 a	0,1063 a
T2 = Vermiculite	83, 3497 a	-6,2531 a	0,1105 a
T3 = Plantmax® Citrus	68, 6207 b	-7,8366 a	0,1212 a

Means followed by the same letter in columns did not differ according to the Duncan's test at 5% significance.

TABLE 2 - Means of total emergence percentage, height, emergence velocity index, and mean emergence time of "araticum-de-terra-fria" (*Annona emarginata* (Schltdl.) H. Rainer) seedlings from seeds subjected to different substrates. *emergence velocity index **mean emergence time.

Treatments/variables	Total mean emergence (%)	height	*e.v.i.	*m.e.t.
T1 = Coconut fiber	76,4 ab	6,76 b	4,7 b	68,5 a
T2 = Vermiculite	87,5 a	7,38 a	8,3 a	61,3 b
T3 = Plantmax® Citrus	68,9 b	5,52 c	5,0 b	63,6 ab

Means followed by the same letter in columns did not differ according to the Tukey's test at 5% significance.

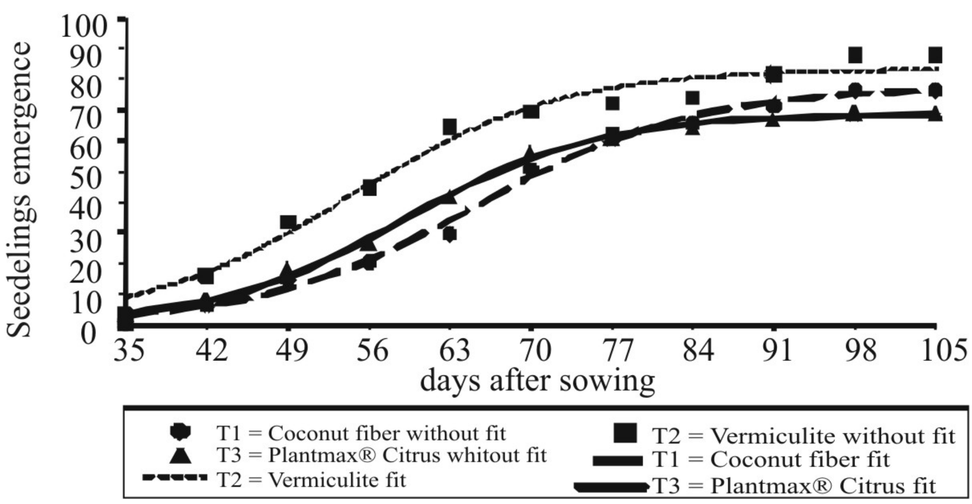

FIGURE 1 – Mean emergence percentage of "araticum-de-terra-fria" (*Annona emarginata* (Schltdl.) H. Rainer) seedlings subjected to different types of substrates for 105 days after sowing, with the original data and those adjusted through the logistic growth equation: y = A / [1 + exp (-(B+Cx))].

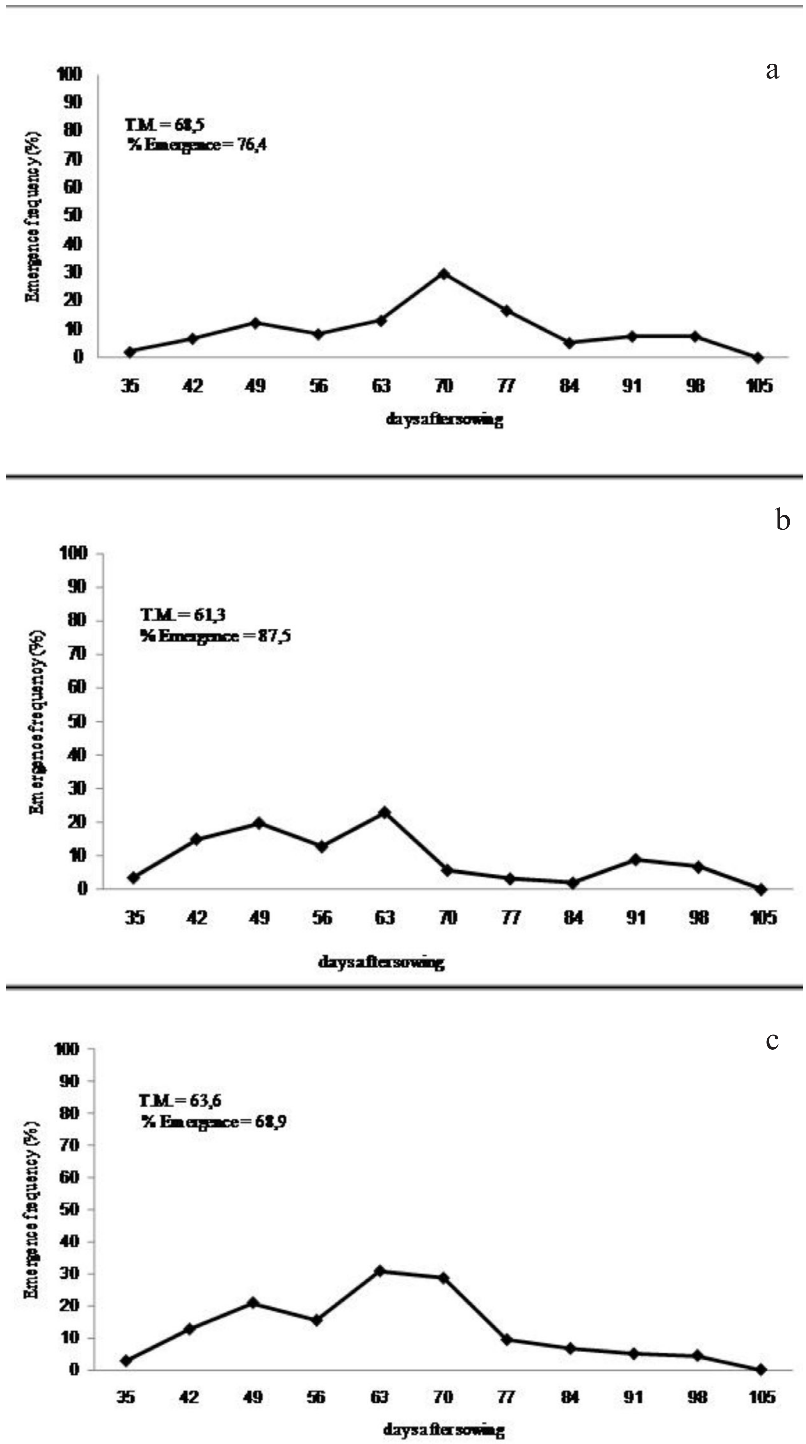

FIGURE 2 - Emergence frequency (percentage) of “araticum-de-terra-fria” (*Annona emarginata* (Schltdl.) H. Rainer) seedlings over time. (a) Emergence frequency by using the substrate coconut fiber. (b) Emergence frequency by using the substrate vermiculite. (c) Emergence frequency by using the commercial substrate PlantMax® Citrus.

CONCLUSION

The substrate vermiculite can be recommended for the initial development of "araticum-de-terra-fria" (*Annona emarginata* (Schltdl.) H. Rainer) seedlings in nursery since it leads to higher emergence percentage and velocity as well as higher development of seedlings.

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