Scientia Agraria Paranaensis – Sci. Agrar. Parana.
ISSN: 1983-1471 – Online

SPENT MUSHROOM SUBSTRATE *Agaricus bisporus* IN THE PRODUCTION OF PEPPER SEEDLINGS

Carlos Godinho de Abreu[1*], Lívia Martinez Abreu Soares Costa[1], Carolina Figueiredo Collela[1], Cibelli Paula de Castro[1], Diego Cunha Zied[2], Eustáquio Souza Dias[1]

SAP 23812 Received: 18/12/2019 Accepted: 20/03/2020
Sci. Agrar. Parana., Marechal Cândido Rondon, v. 19, n. 2, apr./jun., p. 161-167, 2020

ABSTRACT - Mushroom cultivation generates a large volume of SMS (spent mushroom substrate), which needs to be properly discarded to avoid contamination of new production cycles. However, SMS is a rich substrate, and can be used to produce vegetable seedlings. The present study evaluated the feasibility of using SMS of *Agaricus bisporus* as substrate for production of sweet pepper seedlings. The culture substrate was composed of sugar cane bagasse, horse manure, rice straw, soybean meal, chicken bed, urea, potassium chloride, simple superphosphate and gypsum. After cultivation, the SMS was homogenized, wet and composted. The substrate thus processed is the SMS of *A. bisporus*. The substrate Carolina II® was used as control. The following treatments were evaluated: T1- 100% Carolina II; T2 - 25% SMS + 75% Carolina II; T3-50% SMS + 50% Carolina II; T4- 75% SMS + 25% Carolina II and T5- 100% SMS). Seedling and germination characteristics were evaluated. The best germination parameters were observed with the treatment containing 50% of SMS, compared to the commercial substrate. However, for the quality parameters of the seedlings, the best results were obtained with 100% SMS treatment. Therefore, the use of different SMS percentages for the production of pepper seedlings is an alternative to reduce the production cost. The treatment with 100% SMS presented the best DQI values, as it produced vigorous and better quality pepper seedlings.
Keywords: *Capsicum annuum* L., *champignon*, development quality index, SMS.

SUBSTRATO PÓS-CULTIVO DO COGUMELO Agaricus bisporus NA PRODUÇÃO DE MUDAS DE PIMENTÃO

RESUMO - O cultivo de cogumelos gera um grande volume de SMS (substrato pós-cultivo de cogumelos), que precisa ser descartado adequadamente para evitar a contaminação de novos ciclos de produção. No entanto, o SMS é um substrato rico e pode ser usado para produzir mudas de vegetais. O presente estudo avaliou a viabilidade do uso de SMS de *Agaricus bisporus* como substrato para produção de mudas de pimentão. O substrato da cultura foi composto por bagaço de cana, estrume de cavalo, palha de arroz, farelo de soja, canteiro de galinha, uréia, cloreto de potássio, superfosfato simples e gesso. Após o cultivo, o SMS foi homogeneizado, úmido e compostado. O substrato assim processado é o SMS de *A. bisporus*. O substrato Carolina II® foi utilizado como controle. Os seguintes tratamentos foram avaliados: T1 - 100% Carolina II; T2 - 25% SMS + 75% Carolina II; T3-50% SMS + 50% Carolina II; T4- 75% SMS + 25% Carolina II e T5- 100% SMS). As características das plântulas e germinação foram avaliadas. Os melhores parâmetros de germinação foram observados com o tratamento contendo 50% de SMS, comparado ao substrato comercial. Porém, para os parâmetros de qualidade das mudas, os melhores resultados foram obtidos com o tratamento 100% SMS. Portanto, o uso de diferentes porcentagens de SMS para a produção de mudas de pimenta é uma alternativa para reduzir o custo de produção. O tratamento com SMS 100% apresentou os melhores valores de IQD, pois produziu mudas de pimenta vigorosas e de melhor qualidade.
Palavras-chave *Capsicum annuum* L., *champignon*, índice de qualidade de desenvolvimento, SMS.

INTRODUCION

The mushroom cultivation results in a large volume of spent mushroom substrate (SMS), which needs a cure condition for pest and disease recurrence problems, as well as contamination of watercourses. In this context, an alternative could be its use as fertilizer or soil conditioner (RINKER, 2017). However, considering its rich chemical composition and good physical properties, the SMS can have an even more noble destination, and can be used in the substrate composition for the production of vegetable seedlings (MARQUES et al., 2014; LOPES et al., 2015).

The substrates available in the market for the production of vegetable seedlings have good properties, providing seedlings of quality. However, these substrates are costly in the context of family farming, directly impacting their final gain, thus justifying the search for alternative sources (SILVA et al., 2007).

The use of organic residues originating from the agricultural activity and of easy access has been quite

[1]Universidade Federal de Lavras (UFLA), Departamento de Biologia (DBI), Lavras, Minas Gerais, Brazil. Email: carlosgodinhoab@gmail.com. *Corresponding author.
[2]Universidade Estadual Paulista (UNESP), Escola de Ciências e Tecnologia Agrícola (FCAT), Dracena, São Paulo, Brazil.

approached in this type of study (RODRIGUES et al., 2015). Therefore, SMS can be an important source of alternative substrate for seedling production. In addition to the physicochemical properties inherent to a seedling production substrate, the SMS also has substances derived from the fungal metabolism, which can bring additional benefits. One of these benefits may be the production of compounds that act as inductors of resistance in the plant, which have been studied in different species of basidiomycetes (SILVA et al., 2013).

The SMS of the button mushroom (*Agaricus bisporus)* is one of the best options for this purpose because, besides being the most abundant in Brazil, it is a more stable substrate when compared to the SMS of other mushrooms such as shiitake. This is because the substrate is obtained by a composting process that lasts for about 30 days and then goes through the colonization and cultivation processes, which amounts to 3 to 4 months (FIGUEIREDO and DIAS, 2014). In addition, it must consider the fact that the SMS of the *Agaricus* mushrooms come accompanied by soil from the casing layer, which it is a layer of soil 5cm thick applied on top of colonized compost (PARDO-GIMENEZ et al., 2012). Therefore, the *Agaricus* mushroom SMS consists, in fact, of a mixture of compost and soil, which certainly makes it quite interesting as a substrate for the production of vegetable seedlings. Therefore, in this work, the use of *A. bisporus* SMS, in different proportions, was evaluated in the production of sweet pepper seedlings, in comparison to commercial substrate Carolina II®.

MATERIAL AND METHODS

The SMS of *A. bisporus* was provided by the company Sítio dos Micélios, Barbacena (Minas Gerais State, Brazil). The mushroom compost was prepared using sugar cane bagasse, horse manure, rice straw, soybean meal, chicken bed, urea, potassium chloride, simple superphosphate and gypsum. After cultivation, the SMS (consisting of the compost and casing layer) was homogenized, wet and composted for 15 days, with turnings every two days. Finally, the thus obtained substrate was sieved to remove clods and larger fragments of compost, so as to obtain a more uniform granulometry material. The *A. bisporus* SMS thus processed was the substrate use during the present work and the substrate Carolina II® (Carolina Soil do Brasil Ltda) was used as control and in combination with SMS. The composition Carolina II® substrate consists of: moss peat (Sphagnum), expanded vermiculite, charcoal rice husk, dolomitic limestone, agricultural gypsum and traces of NPK fertilizer. The following treatments were evaluated: T1 - 100% Carolina II; T2 - 25% SMS + 75% Carolina II; T3 - 50% SMS + 50% Carolina II; T4 - 75% SMS + 25% Carolina II and T5 - 100% SMS).

The pepper seeds (cultivar Cascadura IKEDA - Agristar do Brasil Ltda) were seeded on the substrates about 1 cm deep in styrofoam trays of 128 cells and taken to the greenhouse where the minimum average temperature was 12ºC and the maximum average of 37ºC. The experiment was carried out in a randomized block design with five treatments and five replicates.

The germination was evaluated from the 1st day after sowing, during 25 days. Percent Germination (G) was calculated by equation 1:

$$\mathrm{G} = \left(\frac{\mathrm{N}}{80}\right) \mathrm{x}\, 100 \qquad \text{(Equation 1)}$$

Where:
N = number of germinated seeds (%).

Germination speed index (GSI) was calculated by equation 2:

$$\mathrm{GSI} = \Sigma\,(\mathrm{ni}/\mathrm{ti}) \qquad \text{(Equation 2)}$$

Where:
ni = number of seeds germinating at time ‘i’,
ti = time after test installation and
i = 1 → 25 days.

Mean germination time (MGT) was calculated by equation 3:

$$\mathrm{MGT} = (\Sigma \mathrm{niti})/\Sigma \mathrm{ni} \qquad \text{(Equation 3)}$$

Where:
ni = number of germinated seeds per day,
ti = incubation time and
i = 1 → 25 days.

Mean germination speed (VMG) was calculated by equation 4:

$$\mathrm{VMG} = 1/\mathrm{t} \qquad \text{(Equation 4)}$$

Where:
t = average germination time (CARVALHO et al., 2009).

After 34 days of sowing, the following seedlings characteristics were evaluated: root length (RL) (mm), shoot length (SH) (cm), plant height (H) (cm) and stem diameter (SD) (mm); root dry mass (RDM), dry shoot mass (SDM), total dry mass (TDM) (sum of RDM and SDM), in grams; number of leaves (NL) and Dickson quality index (DQI).

Finally, the development quality index (DQI), calculated according to the formula (DICKSON, 1960), was calculated by Equation 5:

$$DQI = \frac{(TDM)}{\left(\frac{SH}{SD}\right)+\left(\frac{SDM}{RDM}\right)} \qquad \text{(Equation 5)}$$

For the evaluation of the variables in the molting phase, ten seedlings of each treatment were removed, constituting an experimental unit. Data were submitted to regression analysis and the Skott-Knott test, at 5% probability using the SISVAR program

(FERREIRA, 2011).

RESULTS AND DISCUSSION

For the germination parameters, the best results oscillated between the treatments with 25 to 75% SMS. The highest germination rate and the highest GSI occurred in the substrate containing 50% of SMS (Figures 1 and 2), whereas for VMG and MGT, treatments with 25, 50 and 75% showed the best results indistinctly (Figures 3 and 4). It was observed that, in general, the inclusion of SMS tended to improve the germination parameters to a certain extent, when this effect became negative. That is, if only the germination parameters were considered, the best treatment should be 50% SMS (spent mushroom substrate).

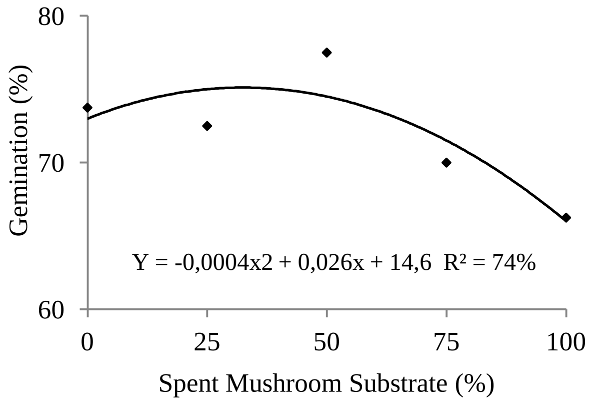

FIGURE 1 - Germination of seeds of *Capsicum annuum* pepper seeds, according to the proportion of SMS.

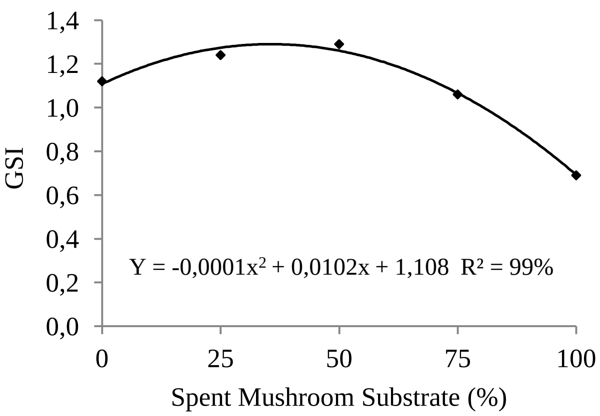

FIGURE 2 - Germination speed index (GSI) of *Capsicum annuum* pepper seeds, according to the proportion of SMS.

The speed of germination is related to the imbibition of water by the seed. The texture (particles) and structure (particle aggregation) of the substrate influence this process through water retention. Some studies show a higher water retention in finer-textured soils (KIEHL, 1979). As mentioned previously, the *A. bisporus* SMS is not only mushroom compost, but a mixture of compost, soil and limestone (FIGUEIREDO and DIAS, 2014). This composition probably favors a higher retention of water and nutrient availability, when SMS is included in the substrate for seedlings production.

Lima et al. (2010) evaluated the effect of different substrates the germination index and seed vigor in the production of *Sicana odorifera* seedlings. However, the best results were obtained with the commercial substrate, contrary to what was observed in the present work. This demonstrates the need for care in the use of alternative substrates, since the economy provided by a cheaper substrate may not result in the best cost/benefit ratio at the end of the process.

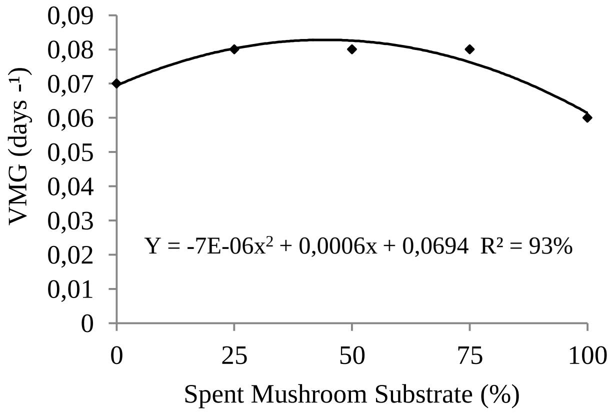

FIGURE 3 - Average speed of germination (VMG) of *Capsicum annuum* pepper seeds, according to the proportion of SMS.

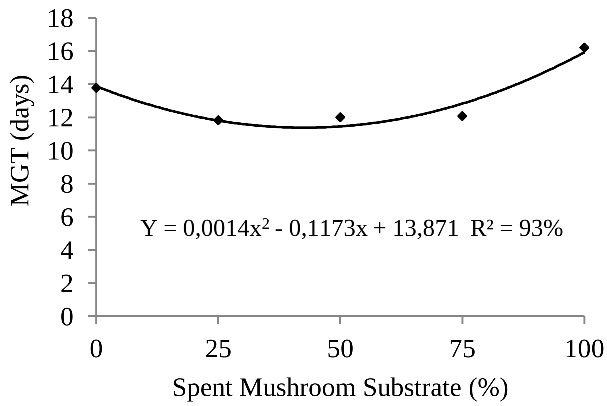

FIGURE 4 - Mean time of germination (MGT) of *Capsicum annuum* pepper seeds, according to the proportion of SMS.

On the other hand, for the DQI, it was observed that the intermediate proportions of SMS did not differ from the commercial substrate. The treatment with 25% of SMS had a significantly lower result, while those of 50 and 75% presented similar results to the commercial substrate. However, for the treatment with 100% SMS, the DQI was significantly superior to the commercial substrate and to the other treatments. Therefore, these results lead to the paradox of having to choose between the best germination parameters, in particular germination percentage and germination time, and the final quality of the seedlings. A lower percentage of germination could mean higher cost on the seed, especially when using top quality seeds, which are also of higher price. However, in this context, it should be noted that the observed differences were not significant (Table 1).

For the mean germination time, the differences were significant, reaching a difference of up to 4 days between the treatment with 100% SMS and the other treatments. This would mean the need for a longer time to obtain the seedlings in case only SMS is used as the substrate for the production of pepper seedlings.

A known negative aspect of *A. bisporus* SMS is its salinity, indicated by the high electrical conductivity of this substrate. This salinity is associated with the presence of fertilizers in the formulation of the cultivation substrate of the mushroom. Due to this problem, a composting step was performed after obtaining the SMS, in order to stabilize the compost and reduce the salinity. Therefore, after the processing, the SMS had an electrical conductivity of 1.98 mS cm^{-1}, which was higher than that normally found in commercial substrates, but within acceptable limits for the production of seedlings.

At first, the higher salinity of the SMS in relation to the commercial substrate could explain why 100% SMS showed germination indexes lower than those observed for the treatments corresponding to the mixtures of SMS and commercial substrate. Probably, promoted a salinity dilution effect. The effect of salinity on the germination parameters occurred from values greater than 2.5 mS cm^{-1}. Therefore, the value of electrical conductivity found in the SMS (1.98 mS cm^{-1}) is within the range considered appropriate by Demontiêzo et al. (2016).

Lopes et al. (2015) observed a negative effect of the inclusion of the SMS of *A. subrufescens* on the substrate of tomato seedling production. A possible explanations presented by the authors for this negative effect was the high salinity level. The authors did not use the same SMS processing procedure described in the present work and, therefore, the SMS of *A. subrufescens* showed an electrical conductivity of 5.51 mS cm^{-1}. In the present work, in addition to another species (*A. bisporus*), the composting process of the SMS certainly contributed to soften the problem of the high salinity observed in this substrate.

The search for alternative substrates to the commercial substrates has a long history, especially with the purpose of reducing the seedlings production costs. Smiderle et al. (2001) proposed a combination of commercial substratum Plantmax® with soil and or sand to produce lettuce, cucumber and sweet pepper seedlings. The authors observed that none of the treatments, either the pure Plantmax® or the combinations, provided the best results for all evaluated parameters and for the three vegetables studied. In these cases, the strategy should be to choose a substrate with the best possible indicators, within the desired commercial standards, with the best cost/benefit ratio and that are equivalent or close to the commercial substrate. However, the alternative substrate tested has not always the same results as the commercial substrate.

TABLE 1 - Table of germination, GSI, MGT and VMG of *Capsicum annuum* seeds according to the of SMS.

SMS (%)	Germination (%)	GSI	MGT (days)	VMG (days)
T1 (0)	73.75 a*	1.12 a	13.77 b	0.07 b
T2 (25)	72.50 a	1.24 a	11.82 a	0.08 a
T3 (50)	77.50 a	1.29 a	12.00 a	0.08 a
T4 (75)	70.00 a	1.06 a	12.07 a	0.08 a
T5 (100)	66.25 a	0.69 b	16.20 c	0.06 c
CV(%)	10.06	16.81	7.72	7.02

*Means followed by different numbers in the lines differ among themselves by Scott-Knott test, at the 5% probability level.

The DQI (DICKSON, 1960) reflects the final quality of the seedling, that is, even if a certain substrate favors better germination conditions, what matters to the producer is the quality of the seedling that he buys or produces. Consequently, although the best germination indexes have a direct effect on the cost of production, the quality of the seedlings reflects directly on its potential for commercialization. Probably, the combination of compost, soil and limestone, found in the SMS, as previously mentioned, contributed to the highest DQI provided by 100% SMS (Table 2).

In this context, it should also be considered that the mushroom substrate is very rich, since its formulation includes, in addition to vegetable raw materials, fertilizers and correctives such as manure, simple superphosphate, urea, limestone and gypsum, besides the inclusion of soybean meal as a source of nitrogen. Therefore, SMS certainly provides a greater contribution of nutrients to the seedlings, compared to the commercial substrate. According to Cerqueira et al. (2015) and Collela et al. (2019), among the factors that interfere in the characteristics of the seedlings is the substrate fertility, which involves components such as nutrients, water, aeration, soil reaction, microorganisms, texture and temperature.

TABLE 2 - Quality index of Dickson (DQI) for pepper seedlings according to the proportion of SMS (spent mushroom substrate).

SMS (%)	DQI
T1 (0)	2,50 b*
T2 (25)	1,39 c
T3 (50)	2,08 b
T4 (75)	2,45 b
T5 (100)	3,71 a
CV (%)	18.96

*Means followed by different numbers in the lines differ among themselves by Scott-Knott test, at the 5% probability level.

Fertility analysis and physicochemical properties showed that the macronutrient concentration of SMS was much higher than the commercial substrate used, except for magnesium (Table 3), which was expected as a function of the nutritional richness of SMS, as discussed previously. Calcium and potassium were found in almost two-fold concentration in the SMS, compared to the commercial substrate.

However, the greatest difference was observed for the phosphorus (P), which was found in a concentration 4.3 times higher than the commercial substrate. In addition, it was also observed that SMS had a lower phosphorus content (Rem-P) than that observed in the commercial substrate. Based on the levels of P and Rem-P, it was found that the relative phosphorus of the SMS was 5.1 times higher than that of the commercial substrate. However, despite being an expressive difference, it is important to note that remaining phosphorus of the commercial substrate was also high, reaching the 'very good' classification (≥ 150%). Therefore, other factors should be considered to explain the effect of SMS on the DQI of the seedlings produced.

TABLE 3 - Results of the fertility analysis and physical-chemical characteristics of the substrates Carolina II® (CII) and SMS of *A. bisporus* used for the production of pepper seedlings.

Samples	pH	K	P	Ca	Mg	Al	H+Al	SB	t	T	BS	m	OM	Rem-P	TN	EC
		---- mg dm^{-3} ---		--------------------- cmolc dm^{-3} -----------------------							----- % -----		dag kg^{-1}	mg L^{-1}	g kg^{-1}	mS cm^{-1}
SMS	6.3	608	542.96	20.4	5.5	0	3.62	27.46	27.46	31.08	88.35	0	15.04	33.07	7.7	1.98
CII	5.5	330	125.75	10.7	5.7	0	4.04	17.85	17.85	21.89	81.55	0	17.16	40.52	trace	0.7

Caption: pH = hydrogen ionic potential, K = potassium, P = phosphorus, Ca = calcium, Mg = magnesium, H + Al = potential acidity, SB = sum of exchangeable bases, t = cation exchange capacity, T = cation exchange capacity at pH 7.0, BS = basal saturation index, m = aluminum saturation index, OM = organic matter, Rem-P = remaining phosphorus, TN = total nitrogen, EC = electric conductivity, trace elements (TEs). Source: Abreu et al. (2019).

Still in the context of fertility, the N-total content, which was found in the value of 7.7 g kg^{-1} in the SMS, is also noteworthy, while only traces were found on the commercial substrate. This difference in nitrogen content is probably one of the major differences in SMS in relation to the commercial substrate in the seedlings production with higher DQI (Figure 5).

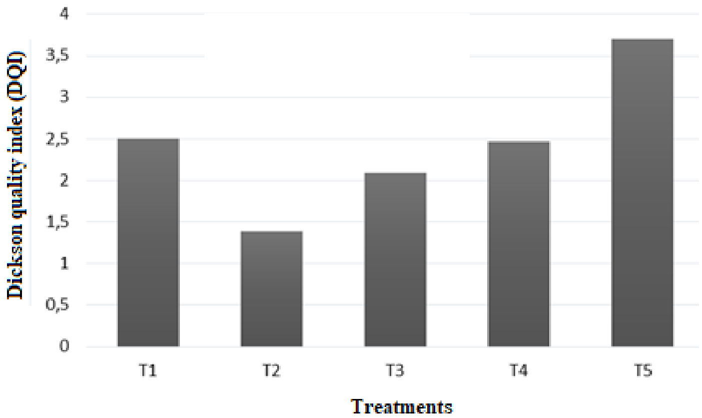

FIGURE 5 - Seed quality index (DQI) of *Capsicum annuum* pepper seeds, according to the proportion of SMS.

Other parameters such as potential acidity, sum of exchangeable bases, cation exchange capacity and base saturation index, presented better results in the SMS. This demonstrates that pure SMS generally has superior chemical and physicochemical properties. One of the few parameters that do not follow this trend is the organic matter content, which can be explained, probably by the presence of soil in the SMS, from the casing layer. The electrical conductivity, as discussed above, is another parameter in which the commercial substrate is superior.

Therefore, with the exception of this last parameter, it is possible to affirm that the SMS presents superior chemical and physicochemical properties.

In addition to all the factors discussed above, there is another one of great importance, but still little known. It is the microbiota present in the mushroom compost and which certainly remains in the SMS after the mushroom cultivation. Of all the microorganisms present, there are certainly species of bacteria that act as growth promoters, as they form part of the rhizosphere of the formed seedlings. As an example, we can cite the evidence that bacteria of the genus *Bacillus* act as elicitors of induction of resistance in the tomato (ROMEIRO et al., 2010).

In previous work, it has been demonstrated that these bacteria are part of the natural microbiota of the *Agaricus* mushroom compost (SILVA et al., 2009). Therefore, in addition to differences in fertility and physicochemical properties, it will be essential to study the SMS microbiota to understand its beneficial effects, when compared to the commercial substrates in the production of vegetable seedlings.

Therefore, considering the superior quality of the *A. bisporus* SMS of further studies should evaluate the pepper production from seedlings produced on SMS substrate. Similarly, SMS must also be tested in the production of seedlings of other vegetable species.

CONCLUSION

The use of different SMS percentages for the production of pepper seedlings is an alternative to reduce the production cost.

The treatment with 100% SMS presented the best DQI values, as it produces vigorous and better quality pepper seedlings.

ACKNOWLEDGEMENTS

To FAPEMIG (Fundação de Amparo à Pesquisa de Estado de Minas Gerais), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) are acknowledged for financial support.

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