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# EFFECT OF BAMBOO POWDER ON SEEDLING DEVELOPMENT OF SELECTED VEGETABLE CROPS

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## ABSTRACT

Bamboo is one of the possible bio-resources to be used in horticultural or nursery planting, although it is still underutilized in Japan or other Southeastern Asian countries. The study sought to utilize bamboo powder as a possible horticultural planting media for greenhouse and nursery as a substitute for peat moss. A series of experiments were conducted on seedling establishment of selected vegetable crops in Tokyo University of Agriculture, Setagaya campus from the autumn of 2022 to the spring of 2023. As a result of bioassays to examine the bamboo powders made of stems with or without branches and leaves, it was found that both of types of bamboo powder could promote seedling development of cucumber. It promoted root system growth which encouraged the biosynthesis of cytokinin and potassium absorption, resulting in significant cotyledon expansion. Bamboo powders also exerted strong allelopathic effect on lettuce, turnip, tomato and carrot seedling development. The fresh bamboo powder was found to release ethyl acetate, a well-known volatile allelochemical. It is recommended that bamboo powder of stem either with or without branches or leaves, be used as a horticultural or nursery planting media after 6 months storage to avoid any adverse effect of ethyl acetate and other water soluble allelochemicals.

**Key words**: allelopathy, cytokinin activity, ethylene production, potassium content, root development

## INTRODUCTION

Bamboo (Bambuseae), naturally growing in Asia, Africa and Latin America, is regarded as one of the most promising biomass resources in future, because of its high growing speed. The stem of bamboo is woody and sometimes reaches up to 40m, but it is categorized as grass family, taking 3-5 years for maturity which is quite short compared to other timber plants which usually need 10-20 years before usage (Shah et al. 2021). Bamboo is known as the fastest growing plant in this planet with the recorded growing speed of 91cm per day (Le 2014). In Japan, however, abandoned bamboo forests are regarded as a serious problem, inasmuch as it can invade surrounding lands and damage both ecosystem and environment (Suzuki 2020). The area of bamboo forests in Japan was reported as large as approximately 170,000 hectares and continues to increase in 2017 (Forestry Agency 2018). We intend to utilize bamboo as a possible horticultural planting base for greenhouse and nursery as a substitute of peat moss. Although peat moss has played an important role as a key component of soil free substrate for greenhouse and nursery usage, the increasing expense and transportation fee, negative impacts of peat mining on wetland ecosystem, and continued trend of weaker yen and stronger dollar require the development of domestic alternatives such as bamboo (Margenot 2018). Reusable or recyclable local materials, derived from renewable resources, should be preferred worldwide (Chrysargyris et al. 2013; Mahmoud et al. 2014; Ceglie et al. 2015).

In this study, the physico-chemical properties of a bamboo powder product were evaluated and the effect on the seedling development of selected vegetables, aiming to show the possibility of utilizing this underestimated plant resource, which might also be widely available in Southeast Asian countries. The possible mechanism of growth promotion effect, especially observed in cotydedon extension of cucumber, was discussed from the hormonal viewpoint.

## MATERIALS AND METHODS

**Chemical analysis of bamboo powder.** Two types of bamboo powders were prepared from 2-3 years old Japanese Moso cultivar (*Phyllostachys edulis* (Carrière) Houzeau) grown in Kagawa Prefecture; one with only fresh mature bamboo stems crushed with a bamboo mill (Houzumi Gokin Co., Ltd., Hyogo, Japan) immediately after removing branches and leaves, and the other with stems including branches and leaves which were simultaneously crushed by a woodchipper GS401D (Ohashi Co., Ltd., Saga, Japan), and passed through an 8mm sieve. Both bamboo powders were immediately stored in a deep freezer (NF-75HC, Nihon Freezer Co., Ltd., Tokyo, Japan) at -40℃. Hereafter, bamboo powder prepared by bamboo mill (made of bamboo stems without branches and leaves) is called “BM” and the one prepared by GS401D (made of bamboo stem with branches and leaves) is called “GS”.

The measurement of pH and EC were conducted as follows: 50 ml of deionized water was added to 10 ml of bamboo powders, and after shaking for 1 hour, pH was measured with LAQUA-PH-SE (Horiba Co., Ltd., Kyoto, Japan) and EC were measured with LAQUAtwin (Horiba Co., Ltd., Kyoto, Japan).

Dried bamboo powders were used for the determination of total nitrogen, total carbon, and inorganic chemical composition. The bamboo powders were dried in a dryer (FO-60P, Tokyo Garasu Kikai Co., Ltd., Tokyo, Japan) at 60℃ for 48 hours and ground into fine powder. Automatic high sensitive NC analyzer SUMIGRAPH NCH-22F (Sumika Analysis Service, Osaka, Japan) was used to measure total nitrogen and total carbon.

To measure inorganic chemicals, 0.5 g of bamboo powder was weighed accurately, placed in a polypropylene container (50 mL DigiTUBEs, GL Sciences, Inc., Tokyo, Japan), and after adding 5 ml of nitric acid (Kanto Kagaku), it was allowed to stay overnight. The container was placed in a heat block (DigiPREP LS, GL Sciences, Inc., Tokyo, Japan) at 100℃ for 60 minutes. After cooling, the volume of the extract was adjusted to 50 ml with deionized water, filtered through filter paper No. 5B, and inorganic components were measured by ICP emission spectrometry (ICPE 9820, Shimadzu, Tokyo, Japan).

**Effect of water-soluble extract of bamboo powders on cucumber seedling development.** The bamboo powders mentioned above (BM and GS) were used for the experiment, and a variety of extract solution were prepared with different dilution ratio as follows. First, deionized water was added to each bamboo powder at a volume ratio of 50:1 (deionized water: bamboo powder), and after shaking for 1 hour, bamboo powder was removed through the filtration with cotton gauze (50x extract solution). The 100 times diluted extract solution was made by adding the same volume of deionized water to this extract solution (100x extract solution). In case of 500 times diluted extract solution, it was prepared by adding 4 times volume of deionized water to the 100 times diluted extract solution (500x extract solution). Deionized water was used for control.

A series of experiments on seedling establishment were conducted on selected vegetable crops in Tokyo University of Agriculture, at Setagaya campus from the autumn of 2022 to the spring of 2023. Cucumber seeds (cv. Yoshinari, Sakata Seeds Co., Ltd., Kanagawa, Japan) were sown on mesh floats edged with styrofoam which was placed on 200 ml of each extract solution in 300 ml plastic cup. Then all the cups were placed at 25℃ in the incubator (LH-220N Nippon Medical and Chemical Instrument. Co., Ltd., Osaka, Japan). After 12 days, shoot and root developments were observed.

**Allelopathic effect of bamboo powders on four vegetable seedlings.** Bamboo powders of BM and GS were prepared by the same way as mentioned above. Allelopathic effect of bamboo powders was checked by sandwich method (Fujii et al. 2004) using lettuce (cv. Furi Flicker Sakata Seed Co., Ltd., Kanagawa, Japan), turnip (cv. Tokinashi Kobu Kohime (stock), Tohoku Seeds Co., Ltd., Tochigi), tomato (cv. Odoriko, Sakata Seeds Co., Ltd., Kanagawa), and carrot (cv. Harumaki Kinko Gosun, Sakata Seeds Co., Ltd., Kanagawa).

First, 10 mg or 150 mg of each bamboo powder was placed in each hole (diameter 35 mm, depth 18 mm) of a 6-hole multi-dish (Costar), and 5 ml of 0.5% (W/V) agar of 40-45℃ was added. The bamboo powder was fixed in a suspended state. Thereafter, 5 ml of agar was added, so that the bamboo powder was sandwiched between the mixture and solidified thereafter. On this upper agar layer, five vegetable seeds aforementioned were sown per hole with the radicle emerging part inserted into the agar. This process was repeated for 3 holes or 3 times. After being kept under dark conditions at 20℃ for 3 days, the radicle and hypocotyl lengths were measured and allelopathic effect of bamboo powder was evaluated.

Since antioxidant activity of bamboo is known to be derived from ethyl acetate (Mu et al. 2004), ethyl acetate released from each sample was measured using gas chromatography. One gram of GS powder with different storage period (0 days, 1 week, 3 months and 6 months) under 5℃, was placed inside a 9ml test tube and incubated for 24 hours at 25℃ in an incubator (LH-220N Nippon Medical and Chemical Instrument. Co., Ltd., Osaka, Japan). One ml headspace gas was taken by syringe and analyzed by GC-4000 (GL Science, Tokyo) equipped with DB-WAC GC Column (30m×0.25mm×φ0.25µm) and FID. Helium was used for carrier gas at 0.85 Mpa, and the temperatures of injector, column, and detector were set at 230℃, 40℃, and 230℃, respectively. Standard ethyl acetate gases of 0.01, 0.5, 1.0 and 5.0, and 10.0 ppm were prepared by using completely vacuumed 1 liter glass jar, injecting 0.01, 0.5, 1.0,5.0, and 10.0 ml ethyl acetate liquid and then equilibrated with air. The authentic curve between peak area and ethyl acetate concentration showed $y=85667x$ ($R^2=0.9879$).

**Effect of bamboo powders on the cytokinin activity, potassium content and ethylene release of cucumber cotyledons.** Since the significant effect of bamboo powders on cucumber seedlings was observed, the hormonal dynamics were analyzed on cytokinin activity and ethylene release as well as potassium content.

The measurement of cytokinin activity was carried out according to the Fletcher's improved bioassay method (Fletcher et al. 1982). Ten pieces of cotyledons of the 5-day old cucumber seedlings (Tokiwa shinjibai cv., Watanabe Noji Seed company, Chiba prefecture) grown at 28℃ in an incubator (LH-220N Nippon Medical and Chemical Instrument. Co., Ltd., Osaka, Japan) under dark conditions in 200 ml of 50 times diluted extract solution of GM bamboo powder in 300 ml plastic cup were excised under dim green light and placed n a 5 cm Petri dish. All the cotyledons were exposed to 2,000 lux fluorescent light for 1.5 hours, and extraction was done using 3ml of acetone. Absorbance was recorded at 663 nm wavelength using UV-VIS spectrophotometer (Model 2600, Shimadzu, Kyoto, Japan). The standard curve of cytokinin activity was prepared using benzyladenine solution with 0, 0.001, 0.01, 0.1 and 1.0 ppm concentration ($R^2$=0.9606).

The amount of ethylene released was determined by GC-FID. Two pieces of cotyledons were placed in 2 ml Eppendorf tube and incubated for 1 hour at 25℃ (LH-220N Nippon Medical and Chemical Instrument. Co., Ltd., Osaka, Japan) after sealing with silicon cup. One ml headspace gas was taken by syringe and analyzed by GC-FID (GC14B), which was equipped with a Sumpack A column (Shinwa Kako, 2.1m×3.2mmφ, glass column filled with porous poly beads). The injector port, column and FID temperatures were set at 80, 80 and $200^0$C, respectively.

Potassium content was measured using ICP emission spectrometry (ICPE 9820, Shimadzu, Tokyo, Japan).

## RESULTS AND DISCUSSION

**Chemical analysis of bamboo powder.** All the three major nutrients, namely: N, P, and K, were higher in GS (stem with branches and leaves) than BM (stem only), possibly due to the contribution of leaves. Accordingly, the C/N ratio was almost half in GS compared to BM. The water content, pH, EC, N content, C/N ratio, P, and K were higher in the bamboo power of GS than BM, while carbon content was almost same (Table 1). Inasmuch as the pH of bamboo powder was almost near neutral ranging from 5.6-6.4 in our experiment, it will be more applicable than peat moss to a variety of vegetables without modification. In case of peat moss, the pH is acidic ranging from 3.0-4.0 (Lee et al. 2021), thus it reduces the pH of soil.

**Table 1.** Chemical properties of bamboo powder

| Bamboo powder | Water (%) | pH | EC (mS/cm) | C (%) | N (%) | C/N | P (%) | K (%) |
|---|---|---|---|---|---|---|---|---|
| BM (stems) | 34.6 | 5.6 | 0.53 | 48.7 | 0.14 | 349 | 0.04 | 0.68 |
| GS (with leaves) | 39.9 | 6.4 | 0.77 | 47.7 | 0.28 | 168 | 0.08 | 0.80 |

**Effect of water-soluble extract of bamboo powders on cucumber seedling development.** The possibility of bamboo powder utilization for horticultural substrate was examined by evaluating the effect of extract solution on cucumber germination and seedling growth development. The results are shown in Photo 1 and Fig. 1 for BM, and Photo 2 and Fig. 2 for GS.

In the case of bamboo powder (BM), the higher concentration of extract solution promoted both shoot and root development, with significant cotyledon expansion effects. The lower the extract solution concentration, the lesser the growth promotion effect, but adverse effects were not observed in all the tested concentrations.

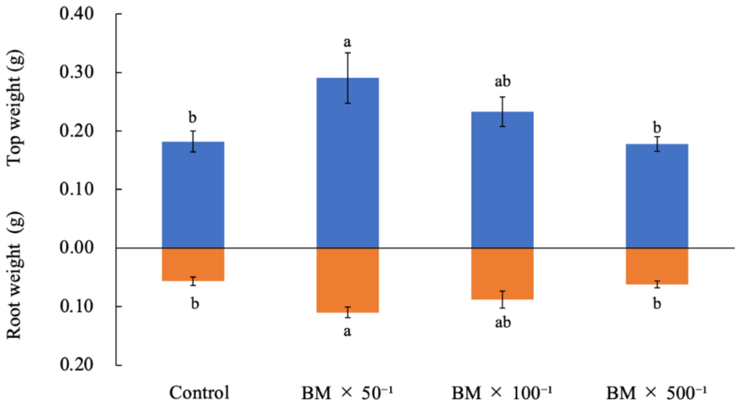



**Figure 1**. Effect of bamboo powder (BM) extract solution on growth of cucumber seedlings
Different letters indicate significant difference at ≤ 0.05 by Tukey's test.

Control BM×50$^{-1}$ BM×100$^{-1}$ BM×500$^{-1}$

**Photo 1.** Effect of bamboo powder (BM) extract solution on growth of cucumber seedlings

On the other hand, bamboo powder (GS), 50 times dilution of extract solution (GS 50$^{-1}$) exerted a strong inhibition effect on cucumber seed germination, though either the adverse nor positive effect of lower concentration extract solution, that is of 100 or 500 dilution treatments, was not observed (Fig. 2 and Photo 2).

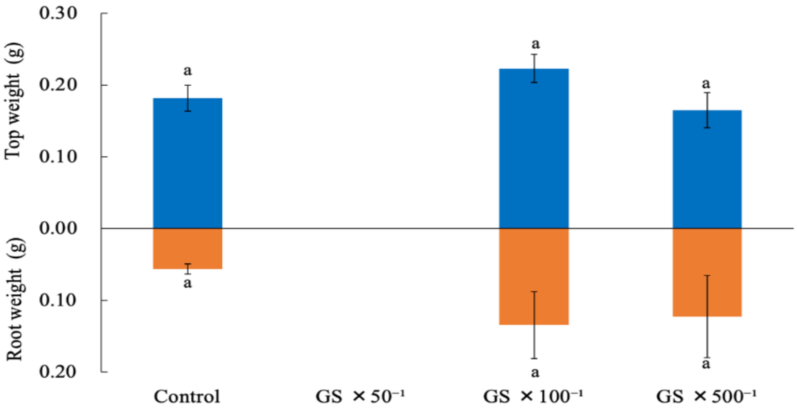



**Figure 2.** Effect of bamboo powder (GS) on growth of cucumber seedlings
Different letters indicate significant difference at ≤ 0.05 by Tukey's test.

Control GS×$50^{-1}$ GS×$100^{-1}$ GS×$500^{-1}$

**Photo 2.** Effect of bamboo powder (GS) on growth of cucumber seedlings

**Allelopathic effect of bamboo powder on four vegetable seedlings.** Because leaves sometimes contain strong allelochemicals and GS is supposed to exert adverse effect on germination or seedling establishment (Kuang et al. 2017; Ogita and Sasamoto 2017), the allelopathic effect of two of these bamboo powders by sandwich method were evaluated. The results were illustrated in Photo 3 and Fig. 3 (lettuce), Photo 4 and Fig. 4 (turnip), Photo 5 and Fig.5 (tomato), and Photo 6 and Fig. 6 (carrot).

In all the tested vegetables, allelopathic effects of bamboo powder extract on shoot and root development were observed in the following order; lettuce ≧ tomato ≧ carrot ≧ turnip. Between the two bamboo powders with or without branches and leaves, that is BM or GS, no clear difference was observed.

It has been reported that the sensitivity of some vegetables was in order as carrot > lettuce > tomato (Hill et al. 2006), but the sensitivity was something inconstant and carrot was more sensitive than lettuce in some cases but vice versa in other cases (Skinner et al. 2012). Likewise, Badmus and Afolayan (2012) illustrated the data showing that radicle is more sensitive than hypocotyl in many cases, but Han et al. (2008) showed that the extract of ginger suppressed the growth of soybean radicle more than hypocotyl, but it suppressed the growth of hypocotyl more than radicle in case of chive. It will be important to consider which kind of crops can be cultivated using bamboo powder materials as growth media.

Control BM GS

**Photo 3.** Effect of bamboo powder extract on lettuce seed germination

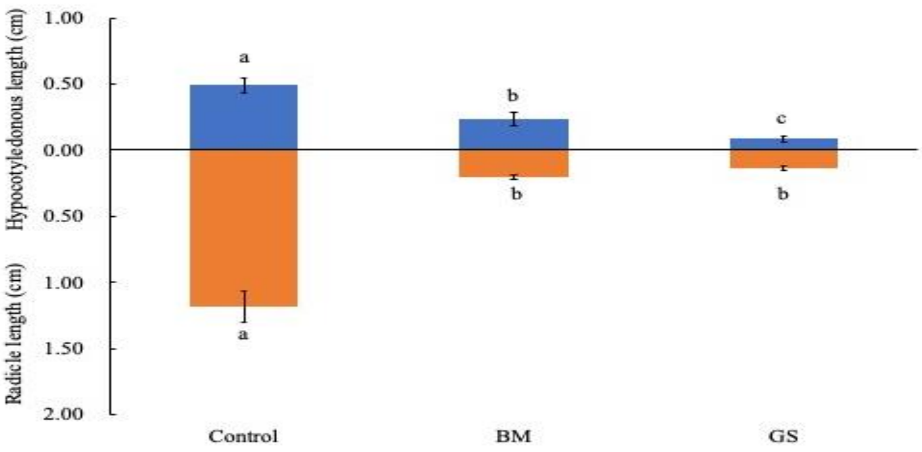



**Figure 3.** Effect of bamboo powder extract on growth of lettuce seedlings
Different letters indicate significant difference at ≤ 0.05 by Tukey′s test.

Control BM GS

**Photo 4.** Effect of bamboo powder extract on turnip seed germination

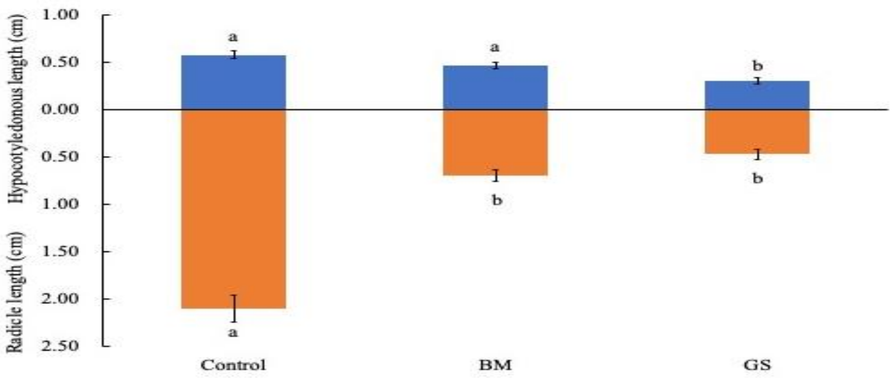



**Figure 4.** Effect of bamboo powder extract on growth of turnip seedlings
Different letters indicate significant difference at ≤ 0.05 by Tukey′s test.

Control BM GS

**Photo 5.** Effect of bamboo powder extract on tomato seed germination

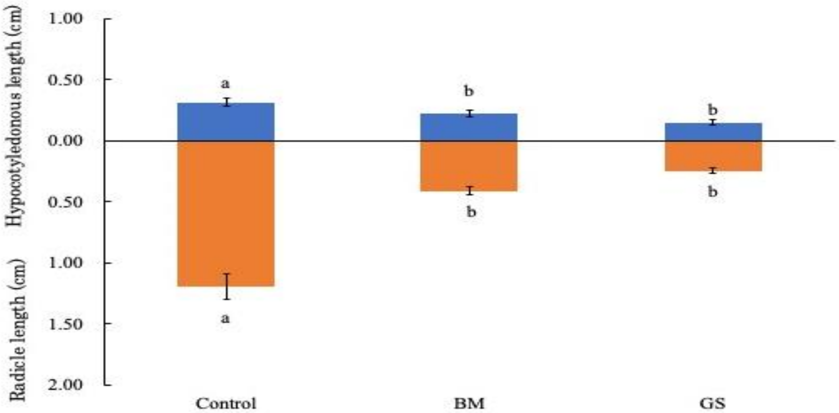



**Figure 5.** Effect of bamboo powder extract on growth of tomato seedlings
Different letters indicate significant difference at ≤ 0.05 by Tukey′s test.

Control BM GS

**Photo 6.** Effect of bamboo powder extract on carrot seed germination

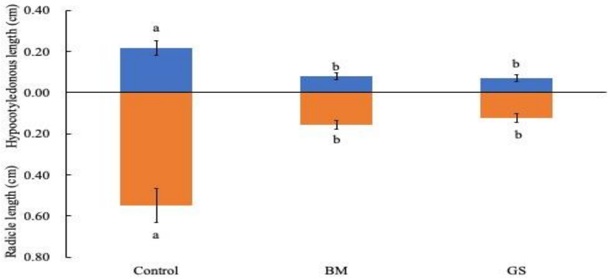



**Figure 6.** Effect of bamboo powder extract on growth of carrot seedlings. Different letters indicate significant difference at ≤ 0.05 by Tukey's test.

Since it is reported that ethyl acetate is one of the main allelochemicals of bamboo (Mu et al. 2004), the concentration of this volatile chemical emitted from the bamboo powder was investigated at different storage periods: 0 day, 1 week, 3 months and 6 months (Fig. 7). The amount of ethyl acetate released from the bamboo powder (BM) was only observed in the freshly harvested sample, and it almost completely disappeared one week later, possibly due to its volatile characteristics. Because another bioassay using sandwich method with lettuce seeds showed that the allelopathic effect of bamboo powder could continue up to 3 months (data not shown), it can be considered that the allelopathic activity of bamboo powder might well be derived from the water-soluble components rather than ethyl acetate.

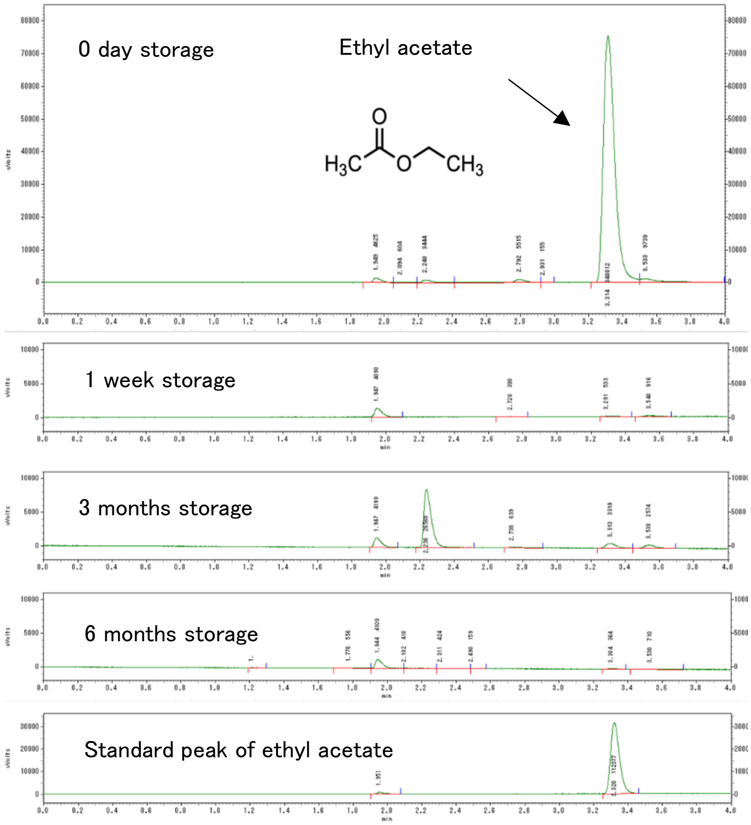
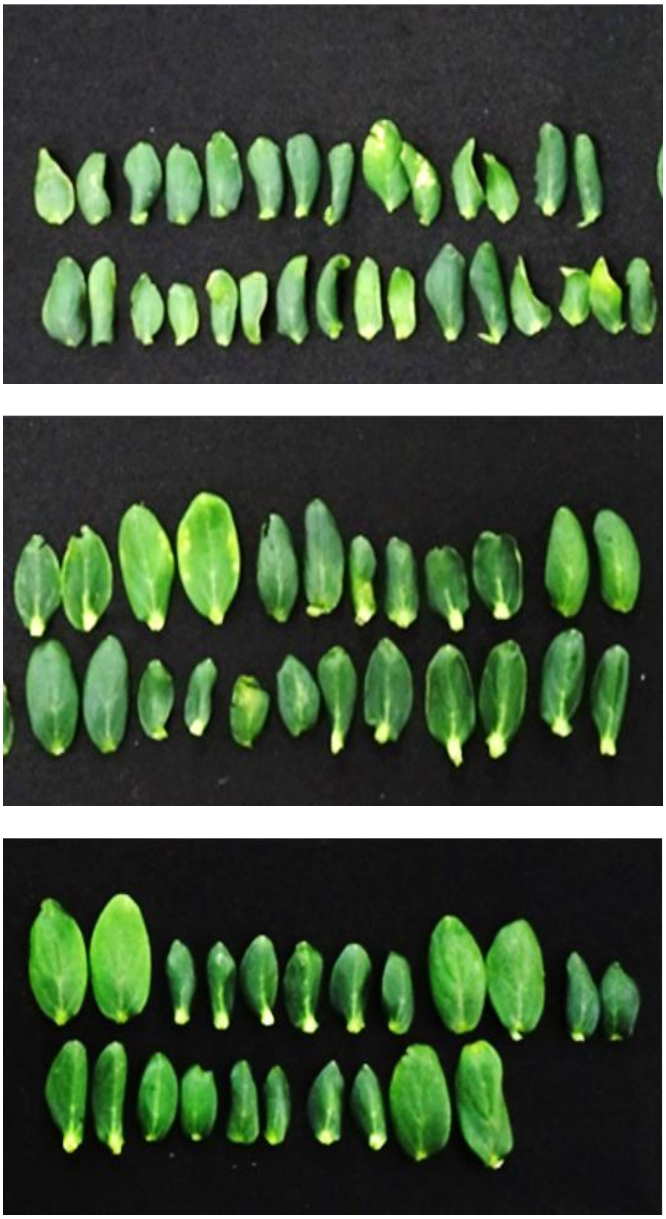



**Figure 7**. Ethyl acetate released from bamboo powder of GS stored for 0 day, 1 week, 3 months and 6 months

**Effect of bamboo powders on the cytokinin activity, potassium content and ethylene release of cucumber cotyledons.** The effect of both bamboo powders (BM and GS) on cucumber seedling growth was statistically significant especially in cotyledon expansion when grown in the extraction solution. Figure 8 illustrates the promotion of cotyledon size of BM and GS treated cotyledons in comparison with control.



Control

BM

GS

**Figure 8.** Effect of bamboo powders (BM and GS) extract solution (100 dilution) on the development of cotyledons of cucumber

The effects of the bamboo powders on cotyledon weight, cytokinin activity shown in benzyladenine equivalent value, ethylene production and potassium content of cucumber cotyledon are shown in Table 2. Cotyledon weight, cytokinin activity and potassium content were significantly increased in both BM and GS, but only GS had significant effect on ethylene production.

These observations suggest the positive relationship between cytokinin, potassium and cotyledon expansion. The utilization of cucumber cotyledon as a bioassay model to check cytokinin activity is highly possible, as cytokinin content and cucumber cotyledon expansion have a linear positive correlation (Esashi and Leopold 1969).

**Table 2.** Effect of bamboo powders on cotyledon expansion, cytokinin activity, ethylene production and potassium content of cucumber seedling.

| **Treatment** | **Cotyledon (mg)** | **Cytokinin activity (BA equivalent) (ng/cotyledon)** | **Ethylene ($C_2H_4$) (pl/cotyledon)** | **Potassium (K) (mg/cotyledon)** |
|---|---|---|---|---|
| Control | 68.1±3.6 b | 0.30±0.0 b | 18.0±1.6 b | 0.4±0.0 b |
| BM | 114.3±5.5 a | 1.08±0.3 a | 21.5±1.2 ab | 3.1± 0.1 a |
| GS | 109.2±4.5 a | 1.13±0.2 a | 24.2±2.3 a | 2.9± 0.1 a |

The promotion effect of seedling development induced by bamboo powder application, either BM or GS, might be derived from the enhanced absorption of potassium encouraged by root system enrichment (Sustr et al. 2019), resulting in the marked cytokinin production ability in the roots (Letham 2019) and finally promoting cotyledon expansion (Esashi and Leopold 1969). There is a synergistic effect of potassium and cytokinin in cucumber cotyledon expansion which promotes ethylene production (Green and Muir 1978; Green 1983). Inasmuch as the cotyledon is reported to play an important role in seedling development, either as an energy storage organ or photo assimilate providing organ (Lovell and Moore 1971), the outstanding cotyledon expansion achieved by bamboo powder application might well contribute to healthy seedling development.

## CONCLUSION

Bamboo powder, which could be provided unlimitedly from the abandoned bamboo forests in Japan, showed a possibility to be utilized as a horticultural or nursery planting media to promote seedling development. Bamboo powders, prepared from stem with or without branches and leaves, enhanced root development, increased cytokinin and potassium content in cotyledon, leading to significant cotyledon expansion. In the case of bamboo powder extract application, the higher concentration of the extract solution induced allelopathic growth retardation in the tested four vegetable seedlings, so stored bamboo powder is recommended for practical usage.

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