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Research Article

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ZEA MAYS STEM AGRICULTURAL WASTE FOR IN-VITRO SHOOT PROPAGATION OF CLINACANTUS NUTANS

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ABSTRACT

The in-vitro shoot propagation performance of Clinacanthusnutans was observed under influenced of different concentration of Zea mays stem agricultural waste in powder and extracts forms. Phytochemical compounds were analyzed. The 4% extract generated optimum number of leaves and 1% extract was the best concentration to produce highest length of shoot. Thus, the used of Z. mays stem powder and extract effectively improved shoot propagation performance of C. nutans. But, the extract showed faster effect on C. nutansgrowth than the powder. The phytochemical screening showed positive result for the present of flavonoid, saponin and steroid. This result supported and shed light on how the Z. mays stem as additional nutrient contributes to the shoot propagation of C. nutans. This study also plays a vital role as basic study for further on-site large scale field application.

Keywords: Clinacanthusnutans; in-vitro; phytochemicals; shoot propagation; Zea mays stem.

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1. INTRODUCTION

Clinacanthusnutans is a well-known medicinal plant due to its biological and phytochemicals activities. It exhibits an excellent performance in cancer, inflammation, dengue and diuretics treatments [1]. Conventional plant growth stem cutting method is slow and hardly fulfilled the market demands. Consequently, a fast propagation in-vitro tissue culture technique is considered by researchers in order to produce enormous high quality and quantity of plantlets in short time. However, this technique is high in cost due to synthetic hormone and additional chemicals [2]. With this regards, an alternative nutrient source is addressed to enhance shoot propagation and cost reduction.

Various agricultural based materials such as papaya Carica papaya [3], banana Musa acuminate [4], coconut Cocosnucifera[5] leaf litter and sugarcane Saccharumofficinarum[6] are studied. However, these alternative nutrient sources for in-vitro tissue culture are useful products or hardly collected in abundant. To authors' best knowledge, there is no study on Z. mays stem agricultural waste as alternative nutrient source for in-vitro tissue culture. Hence, the abundantly and easy availability agricultural waste of Z. mays stem is adopted in this study due to this local agricultural waste is a waste and has limited application.

Agricultural waste contains natural phytochemical compounds such as alkaloid, flavonoid, saponin, tannin, glycoside, steroid, terpenoid, minerals and vitamins [7]. These compounds play an important role for plant growth particularly in shoot propagation, plant defense against pathogens and nutrient absorption. Phytochemical compounds are detected in silk, husk and pollen of Z. mays. But, there is lack of study in Z. mays stem phytochemicals compounds.

The objectives of this study are to investigate the effect of Z. mays stem effects on in-vitro propagation of C. nutans and to determine phytochemical compounds of Z. mays.

2. METHODOLOGY

2.1.Preparation of Z. mays Stem Powder and Extract

Z. mays stem agricultural waste was collected from local farm. Sample was dried, ground and sieved to 710 nm particle size. This sample is known as Z. mays stem powder in this study.

For extract preparation, an amount 0.04 w/v in g/mL of Z. mays stem powder to distilled

water was incubated for two hours at 200 rpm. The mixture was centrifuged and supernatant was stored at 4 ^{o}C for further experimental design.

2.2.Preparation of Medium

Murashige and Skoog medium (MSO) was prepared. An approximately 0.1 µM 6-benzylaminopurine (BAP) was added. After that, the pH of mixture was adjusted to 5.8 using sodium hydroxide or hydrochloric acid. Next, gelrite powder was added into the mixture and autoclaved. Finally, the mixture was pour into jam jars and the media was left to cool down and solidified.

2.3. Surface Sterilization of C. nutans

Plant C. nutans was collected from nursery. A plant stem was cut into 2 cm length at nodal segment. Then, it was washed with running tap water for five minutes and soaked in Clorox for two minutes. It followed by soaking in 70% ethanol for two minutes and 0.1% mercuric chloride for one minute. Finally, it was rinsed with distilled water for three times and air-dried in laminar hood for further subculture work into prepared solid prepared medium. It was left for four weeks induction of shoots before subsequent step.

2.4.Shoot Induction of C. nutans

Different concentration of 1%, 2% and 4% Z. mays stem powder (w/v) and extract (v/v) were added to medium. Then, subculture of plant from section C into prepared mediums was conducted. The culture was incubated in plant growth incubator chamber under controlled environment 25 ± 2 ^{o}C temperature and photoperiod of 16 hours in dark condition and 8 hours under the light 6000 lux.

Propagation performance was measured through number of shoot, number of leave and length of shoot. The data was collected every week for duration of four weeks. The data was expressed in mean and further analyzed by one-way analysis of variance (ANOVA) and t-test.

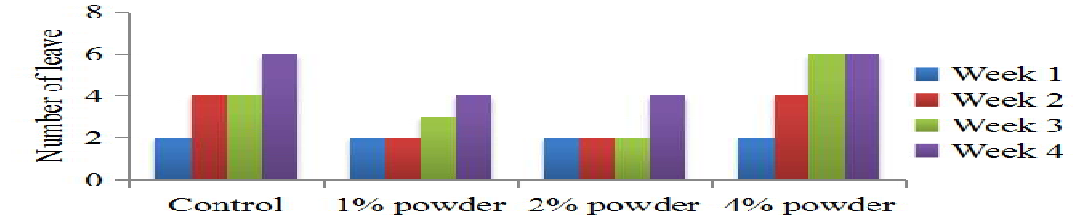
2.5.Phytochemical Compounds Screening

Screening for phytochemical compounds of alkaloid, flavanoid, saponin, tannin and steroid were conducted.

3. RESULTS AND DISCUSSION

3.1.Effect of Z. mays Stem Powder on Shoot Propagation

Fig. 1(a), (b) and (c) illustrate the effects of Zea mays stem powder on shoot propagation during four weeks observation. The 4% powder showed the highest number of leave and length of shoot compared to 1% and 2% powder. The highest number of shoot was found to be at 2% and 4% powder as shown in Fig. 1(b). The 2% powder less performed in leave production because the explants already used the energy consume from the media to generate number of shoot in third week. Meanwhile, the 4% powder showed a significant increase in number of shoot compared to other treatment on week two. Such observation revealed that the effect of Z. mays stem powder for shoot propagation in sequence of shoot number, shoot length and leave number. In [8] reported the similar result on the study about the effect of different plant's powder on growth of pigweed. The result was consistent with this study where it showed that the powder more effective on pigweed shoot compared to pigweed leaf. It can be concluded that higher concentration of powder effectively increased number of shoot and length of shoot of C. nutans.



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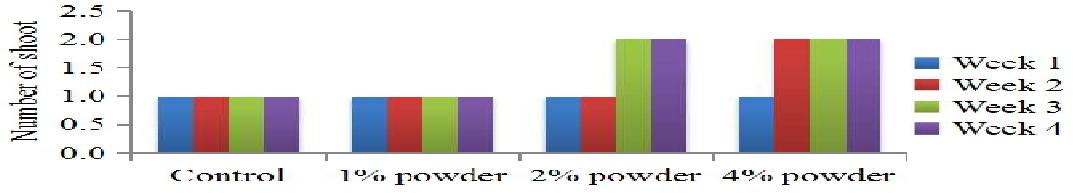

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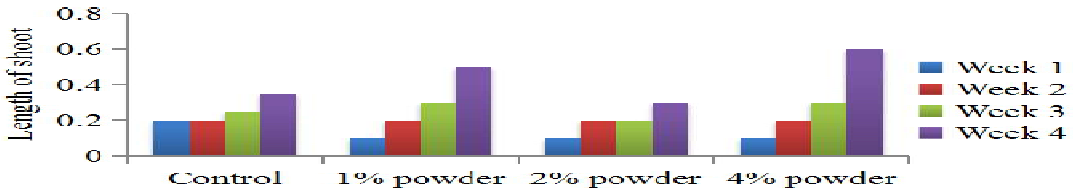


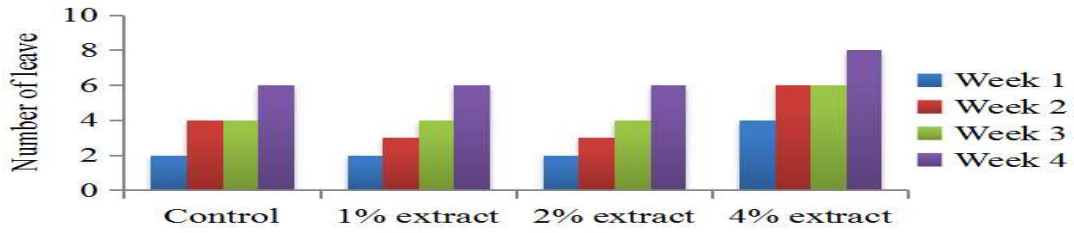
(c)

Fig.1. Effect of different concentration of Z. mays stem powder on C. nutans shoot

propagation

3.2.Effect of Z. mays Stem Extract on Shoot Propagation

The effects of Z. mays stem extract on shoot propagation during four weeks observation are demonstrated in Fig. 2(a), (b) and (c). The 4% extract showed the highest number of leave and number of shoot compared to other treatments. The 1% extract produced the highest length of shoot as shown in Fig. 2(c). It spent most of the nutrient to lengthen the shoot. Thus, it had less energy to produce high number of leave and produced only one shoot. It can be concluded that higher extract concentration was suitable for mass production of leave, the intermediate concentration increased number of shoot and lower concentration suitable to increase length of shoot. In [9] supported this study result where the growth of plant was significantly dose-dependent.



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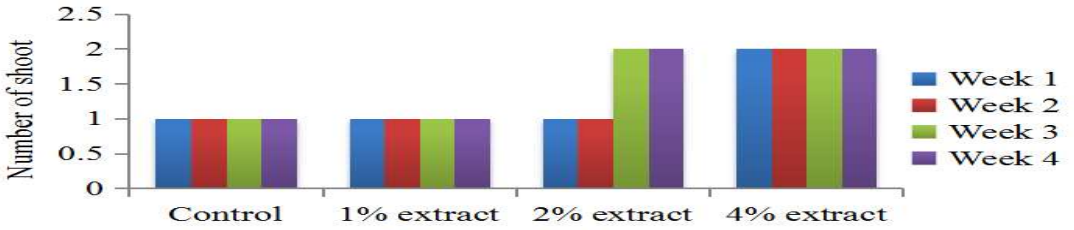

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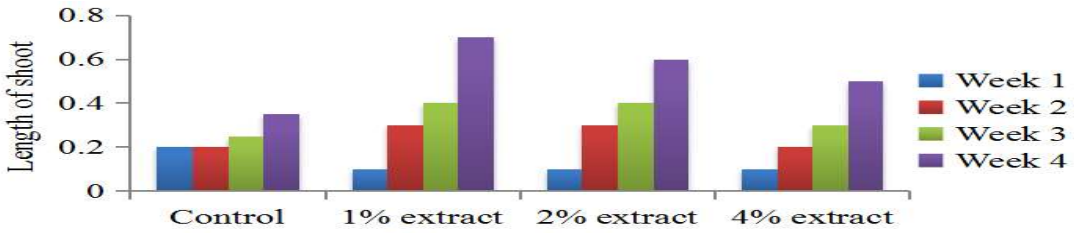

(c)

Fig.2. Effect of different concentration of Z. mays stem extract on C. nutans shoot propagation

3.3.Comparison of Z. mays Stem Powder and Extract on C. nutans Propagation

3.3.1. Number of Leave

Fig. 3 shows the effect of different concentration of Z. mays stem powder and extract on number of leaves. Generally, the highest extract concentration showed the most rapid response on number of leave but powder showed poor performance. There is not significantly different for all treatment in ANOVA analysis ($p>0.05$). However, t-test showed significant different for 4% extract compared to other treatment ($p<0.05$).

In first week, there was an early growth of leave for explants in 4% extract and it showed the highest number of leave compared to other treatments. In second week, there was a growth for explants in 4% powder as well as 1% and 2% extract concentrations. Explants in 1% powder showed growth of leave in third week and 2% powder in the fourth week. The 4% extract showed the highest number of leave and 1% powder and 2% powder was found to be the lowest number of leave in week four. This indicated that explants used most of the energy consumed from the media for shoot development.

The allelopathic compound of phenolic acid, has stimulatory effect on leaf growth. According to [10], Z. mays stem has been reported contain essential compound such as phenolic acid that contributed in growth of leave. Phenolic acid also has high antifungal characteristic, thus it helped in leave development [11]. This result showed similar findings to [12] who reported the Tithoniadiversifolia (Mexican sunflower) extract increased the number of leave and leaf area of cowpea seedlings in tissue culture.

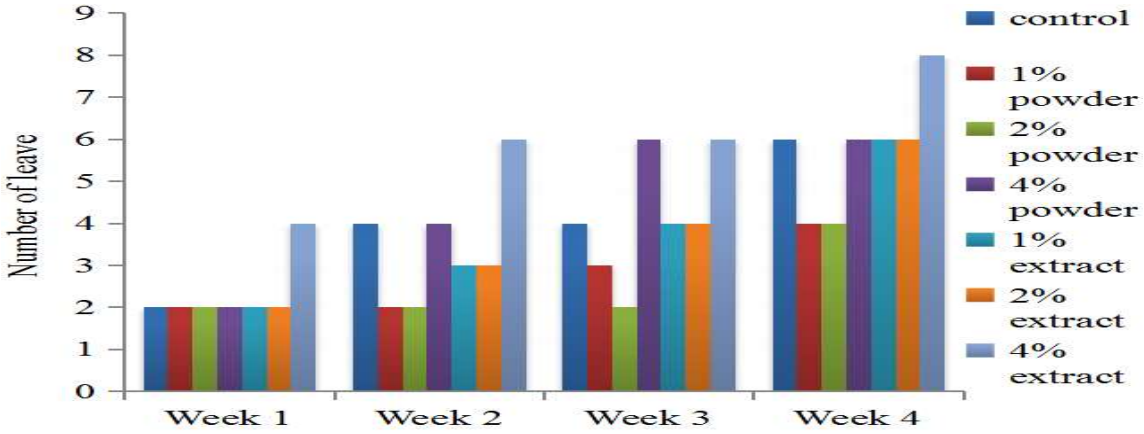

Fig.3. Comparison of Z. mays stem powder and extract on C. nutans number of leave for four weeks

Number of shoot: A slightly increased in number of shoot is shown in Fig. 4. Generally, both Zea mays stem powder and extract performed better in number of shoot propagation than the control. There is significant different between all of the treatment for ANOVA analysis ($p<0.05$). However, statastical analysis showed not significant for t-test ($p>0.05$).

The 4% extract and 4% powder showed the highest number of shoot in second week and the number remained same until fourth week. Explants in 2% powder and 2% extract increased the shoot number from one to two in third week. At high nutrient level condition, explants allocated the high uptake nutrient for growth in number of shoot. The observation revealed generation of new shoot needed high energy consumption, only plant at high level of nutrient can produce new shoot.

The result of this study was supported by the study conducted by [13]. Addition various types and concentrations of algae filtrates not significantly affected shoot's number development.

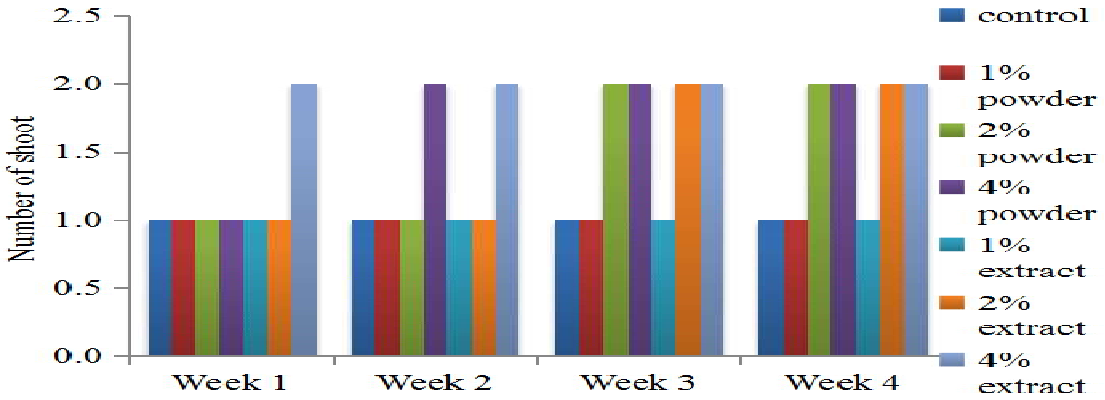

Fig.4. Comparison of Z. mays stem powder and extract on C. nutans number of shoot for four weeks

3.3.2. Length of Shoot

Fig. 5 shows growth of shoot's length in four weeks under effect of Zea mays stem powder and extract. Generally, lower concentration of extract showed better performance in generating length of shoot but powder showed low performance. There is no significant different for ANOVA analysis ($p>0.05$). However, there is significant different for 4% extract compared to other treatment in t-test as $p<0.05$.

There was no increased of shoot length under effect of Z. mays stem powder and extract in first week. There was a slightly increased of shoot length for all concentration in second week.

The explants in 1% extract and 2% extract showed the highest shoot length. In third week, there was an increase of length of shoot for 1% powder, 4% powder and 4% extract. The lengthening of shoot for these explants continued until fourth week. The 1% extract showed the highest length of shoot in fourth week. The 2% powder did not give much effect in length of shoot.

The result showed that low concentration of Z. mays stem promoted length of shoot. This is consistent with [14] who focused on neem leave aqueous extract on shoot length of wild oat. This confirmed that high concentration of extract inhibits length of plant shoot.

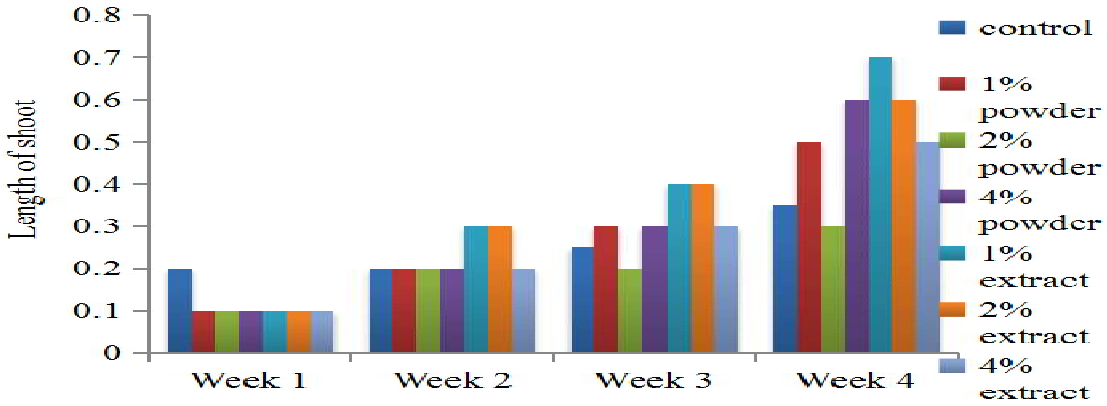

Fig.5. Comparison of Z. mays stem powder and extract on C. nutans length of shoot for four weeks

3.3. Phytochemical Compounds Screening

The phytochemical screening of the Z. mays stem showed positive result for flavanoid, saponin and steroid. But, alkaloid and tannin were not identified in Z. mays stem agricultural waste.

Flavanoid is a phenolic compound that functions in plant development especially in shoot branching and development. It also plays a major role in transportation of plant hormone to all part of the plant and defense mechanism against bacteria. These characteristics enhance cell growth and differentiation. In [15] supported similar findings that the flavanoid caused rapid growth of Eucalyptus urophylla and Eucalyptus grandis hybrid. Furthermore, in [16] reported high flavanoid content in leave of Buteamonospermacontributed in leave development. These has evidenced the observed result reported.

Saponin increases the rate of shoot and leave development. It exhibits antifungal activity, thus

it protects the plant from any infection and allows the plant to grow healthy. In [17] reported that saponin increased growth rate and acceleration of seed germination of cereals and tomato. Therefore, saponin in Z. mays stem supported the observed results in shoot number development.

Steroid improves plant height, leaf morphology and branching of plant. It also stimulates cell division and shoots elongation. Steroid influenced shoot length depends on its concentration [18]. Based on this study, higher concentration of powder and lower concentration of extract were performed better in developing shoot length. It can be concluded that steroid in Z. mays stem contributes in shoot length development as reported.

4. CONCLUSION

This study investigates the effect of Z. mays stem agricultural waste for in-vitro shoot propagation of C. nutans. Two types of treatments, powder and extract were examined in terms of in-vitro shoot propagation of C. nutans. Number of leave, number of shoot and length of shoot were observed for duration of four weeks. Phytochemical analysis for alkaloid, flavonoid, saponin, tannin and steroid was conducted. The Z. mays stem powder showed better performance in number of shoot when compared to length of shoot and number of leave. On the other hand, high concentration of Z. mays stem extract affects number of leave significantly, intermediate concentration for number of shoot and low concentration for length of shoot. For comparison, the Z. mays stem extract exhibited better shoot propagation performance than powder. It was found that plant focused on initiate of shoot number propagation as main activity that high energy consumption is required. Meanwhile, low concentration of Z. mays stem significantly affects length of shoot. Phytochemical compounds of flavanoid, saponin and steroid were identified in Z. mays stem. These compounds contributed in shoot branching and development, thus increased the shoot propagation of C. nutans and supported observed results. The Z. mays stem is a potential agricultural waste for in-vitro shoot propagation application. This study also sheds light for further field application study.

5. ACKNOWLEDGEMENTS

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