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Example format

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Page number from top: 0.5 inch or 1.27 cm, Cordia New 14

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Shell structure investigation and utilization of bio-extract fermented from green mussel shell

1 Enter with Cordia New 14 pt

Ratikorn Smithmaitrie[a,b], Pitcha-orn Sirichewakesron[b], Kanyarat Suwannateep[c,*]

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Abstract (Level I heading: Cordia New 14, Bold)

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…… (Not over than 200 words)……

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Keywords: Bio-extract, Green mussel, Shell, Waste (allow up to 5 words, alphabetical order)

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Introduction (Level I heading: Cordia New 14, Bold)

Green mussels are an economic aquatic marine shellfish in Thailand due to their low price and their various nutritional components, such as protein, vitamins and minerals (Strunk and White, 1979; Morgan, 1999; Chantiratikul et al., 2016; Zhu et al., 2016). The mussels are processed into high value seafood products using different types of processing such as drying, pickling, boiling and freezing)……

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Materials and Methods (Level I heading: Cordia New 14, Bold)

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Preparation of green mussel shell for non-aerated fermentation (Level II heading: First letter of each word is uppercased, Italic, Align-left)

Samples of green mussel shell were collected from a community that processed green mussel in Samut Songkhram and Samut Songkhram province, Thailand. The shells were cleaned of tissue scraps and barnacles and then dried before grinding the shell in pieces approximately 2.5 cm in length (size of shell up to 1 inch).

The composition used in fermentation involved: ground green mussel shell, molasses, water and Microbial activator Super LDD 2. The proportions used were based on Loth (1999). The experiment involving green mussel shell non-aerated fermentation was divided into two treatments with three replications (Table 1): treatment 1, the composition proportions followed the formula of Shewale et al. (2016); and treatment 2, added 60% (by volume) of molasses to treatment 1. The proportions for non-aerated fermentation of green mussel shell are shown in Table 1.

Citation Table in text

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Table 1 Selected phytosterol in rice bran stearine (RBS) from winterizing step, coconut oil (CO) and palm kernel oil (PKO)

use a superscript letter

Type of oil	β-sitosterol (mg/100 g)	Campesterol (mg/100 g)	Stigmasterol (mg/100 g)	Total phytosterol (mg/100 g)
RBS	1508.50±6.36[a]	341.75±9.61[a]	2141.50±149.20[a]	3991.50±146.37[a]
CO	12.11±0.16[b]	1.72±0.28[b]	11.43±0.31[b]	25.26±0.76[b]
PKO	23.16±1.10[b]	1.48±0.08[b]	11.83±0.38[b]	36.47±1.40[b]

1.0 Line spacing

*Means±SD in the same column followed by different superscripts are significantly different ($p < 0.05$)

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use an italicized lower-case letter

Investigation of composition of green mussel shell before and after non-aerated fermentation

The amounts of calcium, chitin and protein in the green mussel shell were analyzed before and after 15 wk of fermentation. Quantification of calcium content in the green mussel shell followed Association of Analytical Chemists (2000). A sample (1 g) of the shell was added to 69% HNO_3 and $HClO_4$ (ratio 1:2) and heated

on a hot plate set up in a fume hood for digestion of the shell. Diluted shell digested solution was made up to 100 mL with deionization water. The wavelength for calcium analysis was 422.7 nm and the radiation of the calcium absorbed was compared with a calibration curve and the calcium content in the shell was shown as a calcium oxide (CaO) value (Palma et al., 2009; Chantiratikul et al., 2016).

Chitin and chitosan were determined according to Strunk et al. (1979) and Isnaeni (2007). A sample of crushed green mussel shell (100 g in 1 cm pieces) was mixed with 1M NaOH (ratio 1:10 weight per volume, w/v) and left on a magnetic stirrer at 100ºC for 4 hr. The sample was filtered, washed with distilled water and adjusted to pH 7 before being dried in an oven at 50ºC for 24 hr. Then, the green mussel shell residue was weighed. The shell residue was added to 0.68 M HCl (ratio 1:10 w/v), soaked for 6 h and then filtered with the reaction with the 0.68 M HCl repeated 5–6 times or until no gas bubbles were produced. After washing the sample with distilled water and adjusting to pH 7 (based on a pH paper test), the pigments were removed by soaking in 99.5% acetone and then in 100% methanol. The soaking was repeated until no color was evident in the solvent. Then, the green mussel shell residue was dried in a hot-air oven at 50°C for 24 hr, weighed and the amount of chitin was determined according to Equation 1:

insert symbol of °

citation equation in text

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$$\% \text{ chitosan} = \frac{\text{Weight of chitosan}}{\text{Weight of chitin}} \times 100 \qquad (1)$$

provide the equation number

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Acid-base titration method

Degree of deacetylation (DD) of chitosan was determine by using Acid-base titration (Dutta and Priyanka, 2022). The DD of each chitosan sample was calculated using the following Equation 2:

$$\% \text{ of DD} = \frac{(v1 - v2) \times 16}{v1 \times 9.94 \times x} \times 100 \qquad (2)$$

where x is the weight of dried chitosan; V1 = volume of chitosan solution prepared in 0.1 N HCl solution in mL; V2 = volume of 0.1 N NaOH in mL; 9.94 is the theoretical value of % NH_2 group content of chitosan

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Statistical analyses

Data were analyzed using one-way analysis of variance with significance tested at $p < 0.05$ (R Core Team, 2014). All statistical analyses were performed using the SPSS software (version 11, SPSS Inc.; Chicago, IL, USA)

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Results and Discussion

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Composition of green mussel shell before and after non-aerated fermentation

Before fermentation, the mean amounts (±SD) of calcium, chitin and protein were 53.97±6.56% CaO%, 26.28±2.12% and 3.58±0.14%, ... are shown in Fig. 1 and Fig. 2

citation figures in text

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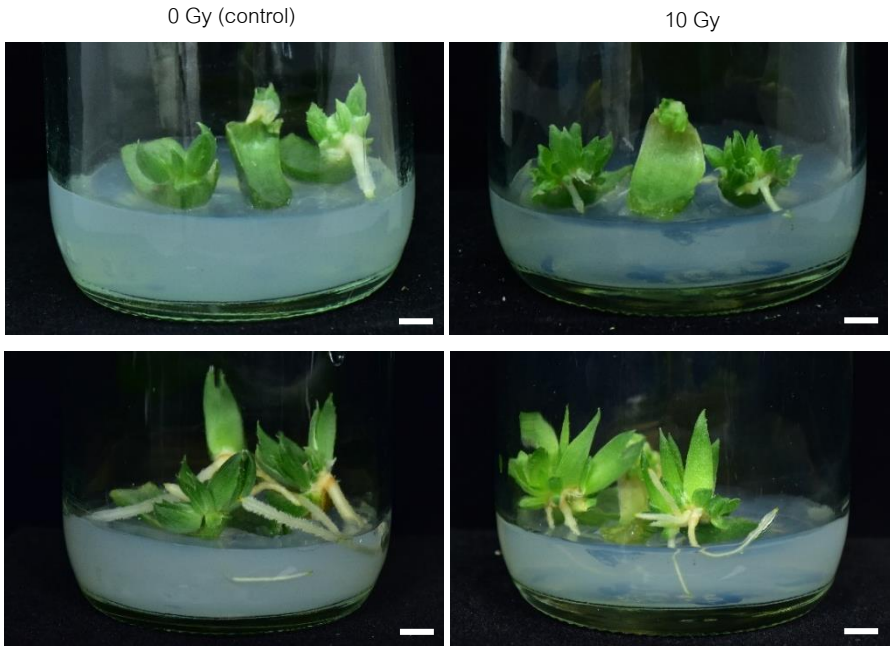

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Fig. 1 Plantlet formation of *H. transiens* after 0 Gy and 10 Gy gamma irradiation and culturing for 30 days and 60 days. Scale bar: 0.5 cm

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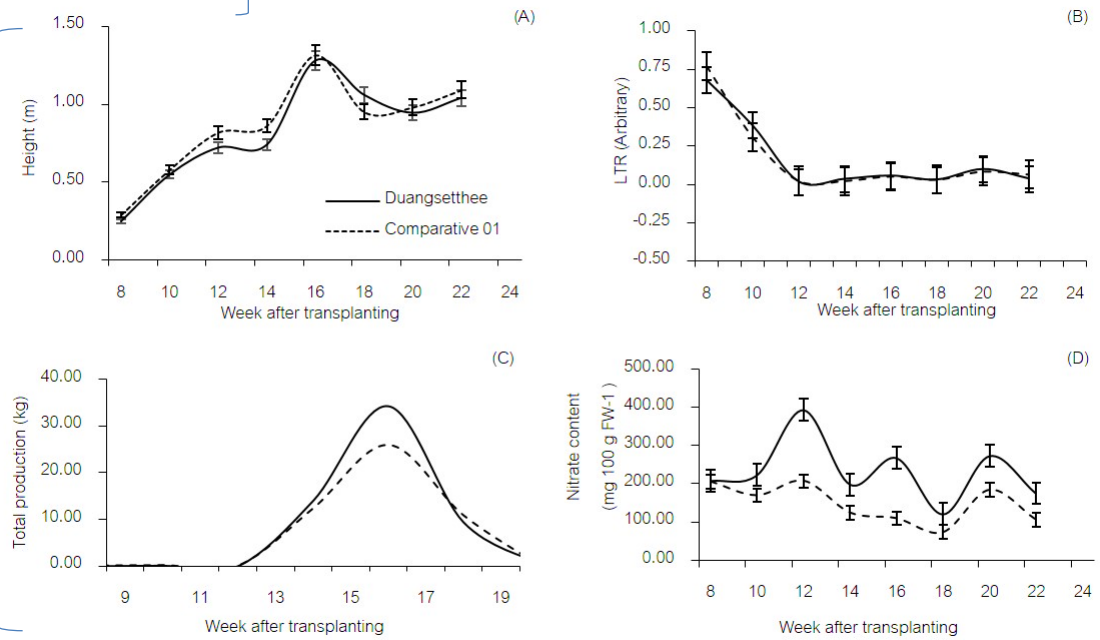

Fig. 2 Physiological parameters change of two hot-pepper cultivars after transplanting in an open field: plant height (A); light transmission ratio (B); fresh production including green and red fruits (C); nitrate content in leaves of two hot pepper cultivars (D)

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Acknowledgements

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Form of number and symbols

1. Numbers and symbols

- Système International (SI) units are used
- Temperatures are given in degrees Celsius, 33°C, 25–30°C
- Liter always has a capital letter: mL/s, µL,
- Superscripted ordinal qualifiers after number: 1^{st}, 2^{nd}, 3^{rd}, 4^{th}
- Use “en dash (–)” for ranges of number such as: 1991–1996, 25–30 °C,
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- Use “insert symbol” function to insert the symbol as: 2.4×610^{-2}, 60°C, 56.09±0.02

2. Statistics

- For *p* value, use an italicized lowercase letter, with a space on either side: $p < 0.05$; $p = 0.562$
- For sample size, use an italicized lowercase, with space on either side: $n = 36$
- Student’s t test not Student’s t-test and no italics and similarly for F test
- SD value no italic
- No space between mean±SD value; 12.11±0.16

Form of in-text citation and References

1. In-text citations

Writing format of the in-text citation as shown in the table below

Type of citation	Citation at the beginning of a sentence	Citation at the end of a sentence
One author	Walker (2007)	(Walker, 2007)
Two authors	Walker and Smith (2004)	(Walker and Smith, 2004)
Three authors or more	Walker et al. (2004)	(Walker et al., 2004)

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2. References

- Authors' name, use this form: Author, A.A., Author, B.B., Author, C.C. year. List author's surname and followed by abbreviation of first name (include middle name).

- Use "et al." for author more than 9 person after the 3rd author name, for example Zhu, F., Chen, J., Xiao, X., et al. 2016.

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- Title name: for book; use capitalize for first letter each word, for article; use capitalize for first letter of first word except special name

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Morgan, L. 1999. Hydroponic Lettuce Production: A comprehensive, Practical and Scientific Guide to Commercial Hydroponic Lettuce Production. Casper publication. Australia.

Wyn, J.R.G., Brady, C.G., Speirs, J. 1981. Recent Advances in the Biochemistry of Cereals. Academic Press. London, UK.

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Form:

Author, A.A., Author, B.B., Author, C.C. Year. Title of Book, ordinal number ed. Publisher Name. Country.

Example:

Association of Official Analytical Chemists. 2000. Official Methods of Analysis, 17th ed. The Association of Official Analytical Chemists. Gaithersburg, MD, USA.

Strunk, Jr., W., White, E.B. 1979. The Elements of Style, 3rd ed. Macmillan. New York, NY, USA.

c. Book chapter in book with editors

Form:

Author, A.A., Author, B.B., Author, C.C. Year. Title of chapter. In: Author, A.A., Author, B.B., Author, C.C. (Eds.). Title of book. Publisher Name. Country, pp. xx–xx.

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Form:

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Example:

Costa, B.H.G., de Resende, M.L.V., Monteiro, A.C.A., Ribeiro Júnior, P.M., Botelho, D.M.D.S., Silva, B.M.D. 2018. Potassium phosphites in the protection of common bean plants against anthracnose and biochemical defence responses. J. Phytopathol. 166: 95–102. doi.org/10.1111/jph.12665

Graf, S., Egert, S., Heer, M. 2011. Effects of whey protein supplements on metabolism: Evidence from human intervention studies. Curr. Opin. Clin. Nutr. Metab. Care. 14: 569–580. doi: 10.1097/MCO.0b013e32834b89da

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Blanchard, M.G., Runkle, E.S. 2006. Temperature during the day, but not during the night, controls flowering of *Phalaenopsis* orchids. J. Exp. Bot. 57: 4043–4049.

Chantiratikul, A., Borisuth, L., Chinrasri, O., Saenthaweesuk, N., Chookhampaeng, S., Thosaikham, W., Sriart, N., Chantiratikul, P. 2016. Evaluation of the toxicity of selenium from hydroponically produced selenium-enriched kale sprout in laying hens. J. Trace Ele. Med. Biol. 35: 116–121.

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Myers, N., Mittelmeier, R.A., Mittelmeier, C.G., da Fonseca, G.A.B. Kent, J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853–858. doi.org/10.1038/35002501

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Pinitpaitoon, S. 2012. Enhancing productivity of red clay soil for maize cropping by organic fertilizer application in combination with mineral fertilizers. Ph.D. thesis, Graduate school, Kasetsart University. Bangkok, Thailand.

4. Proceedings/Research report

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Author, A.A., Author, B.B., Author, C.C. Year. Title. In: Name of proceedings. City, Country, pp. xx–xx.

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Author, A.A., Author, B.B., Author, C.C. Year. Title. City, Country. http://xxxx. Date of searching.

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Nabhitabhata, J., Chan-ard, T. 2005. Thailand Red Data: Mammal, Reptiles and Amphibians. Office of Natural Resources and Environment Policy and Planning. Thailand. https://www.pangolinsg.org/wpcontent/uploads/sites/4/2018/06/Nabhitabhata_Chan_ard_2005_Thailand-Red-Data-Book_Mammals-et-al.pdf, 7 September 2021.

The Thai Tapioca Development Institute. 2019. Harvested Area and Production, Past Years. https://tapiocathai.org/English/L1_e.html, 5 September 2021.

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Example:

Bleeker, P. 1850. Contribution to the knowledge of the ichthyological fauna of Borneo, with description of 16 new species of freshwater fishes. Nat. Tijdschr. Ned. Ind. 1: 1–16. [in Dutch]

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