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EDITORIAL TEAM

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Welcome message from Editor-in-Chief

Dear authors, reviewers, and readers

Our great honor is to present the first issue of the third volume of the Journal of Science and Agricultural Technology (JSAT), the official journal of the Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna (RMUTL), Thailand. The research article still contains five articles from various contributions in this issue. The JSAT has been published in Thai Journal Online (ThaiJO), indexed in Google Scholar, and Digital Object Identifier (DOI) under the National Research Council of Thailand. The journal will publish high-quality articles under an intense peer-review process with solid support from various educational institutions domestically and abroad.

As an Editor-in-Chief, I promise to move forwards to gain international recognition preparing for a higher index ranking. Besides, I strongly encourage researchers around the globe to submit manuscripts to share knowledge and promote the growing field of science and agricultural technology. I am so grateful for the support from our submitting authors, reviewers, and staff. Without you, the success of the current issue would not be possible.

Kind regards,

Assoc. Prof. Dr. Suntorn Wittayakun

Editor-in-Chief Journal of Science and Agricultural Technology
Dean of the Faculty of Science and Agricultural Technology
Rajamangala University of Technology Lanna, Thailand.

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ABOUT THE JOURNAL

Journal of Science and Agricultural Technology (JSAT) publishes original research contributions covering science and agricultural technology such as:

• Natural and applied sciences: biology, chemistry, computer science, physics, material science and related fields. Papers in mathematics and statistics are also welcomed, but should be of an applied nature rather than purely theoretical.

• Agricultural technology: plant science, animal science, aquatic science, food science, biotechnology, applied microbiology, agricultural machinery, agricultural engineering and related fields.

Furthermore, the JSAT journal aims to span the whole range of researches from local to global application.

The JSAT is published two issues a year.
Issue 1: January - June
Issue 2: July - December

Submissions are welcomed from international and Thai institutions. All submissions must be original research not previously published or simultaneously submitted for publication or submitted to other journals. Manuscripts are peer reviewed using the double-blinded review system by at least 3 reviewers before acceptance. There is no publication or processing fee.

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CONTENTS

JSAT IVCST 2021
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Land surface temperature estimation for Buriram town municipality, Thailand

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ABSTRACT

Land Surface Temperature (LST) has long been monitored and studied; however, the most reliable method of estimating the LST has yet to be examined regarding the mixed land-use types over a small city. This research explores an optimum method for Land Surface Temperature (LST) estimation in a city using the data from LANDSAT-8. Four favored LST retrieval approaches, the Radiative Transfer Equation-based method (RTE), the Improved Mono-Window method (IMW), the Generalized Single-Channel method (GSC), and the Split-Window algorithm (SW), were used to estimate the LST over Buriram Town Municipality, Thailand. The calculated LST from these four methods was compared with ground-based temperature data of 100 measured sites over the study area on the same date and time as the employed Landsat-8 data. The lowest Normalized Root Means Square Error (NRMSE) was considered to identify the optimum method of the LST estimation. The SW algorithm provides the lowest NRMSE value (0.114), followed by the RTE (0.171), the IMW algorithm (0.181), and the GSC (0.219). As a result, the SW algorithm is the optimum method in LST estimation for Buriram Town Municipality. The SW algorithm mainly eliminates atmospheric effects based on differential absorption in two thermal bands, which have shown the smallest error in the retrieval of LST. The explored optimal method will benefit GIS specialists working for Buriram local government to conduct the best practice to monitor the LST over the city. The other local governments could consider the SW algorithm to monitor the LST over their small cities with similar contents.

Keywords: split-window, Landsat-8, land surface temperature, thermal bands, Buriram

INTRODUCTION

Land Surface Temperature (LST) is an essential variable for estimating radiation and energy budgets associated with mainland surface processes (Holmes et al., 2013; Orhan et al., 2014; Wu et al., 2013). Knowledge of LST distribution can provide useful information about the surface's physical properties and climate, which have a role in a variety of fields, including land-atmosphere energy budget (Weng and Fu, 2014; Wu et al., 2015), climate change (Avdan and Jovanovska, 2016; Weng et al., 2014; Wu et al., 2015), hydrological cycle (Avdan and Jovanovska, 2016; Bendib et al., 2016; Wu et al., 2015), evapotranspiration (Bendib et al., 2016; Weng et al., 2014), and urban climate (Bendib et al., 2016; Mechri et al., 2014; Weng and Fu, 2014). LST data from satellite remote sensing provides denser spatial sampling intervals than those taken at ground sites (Zhou et al., 2015). Data from the LANDSAT series is widely used for retrieving the LST, according to the downloadable free data from the USGS website, regular revisit times, and long-term recorded data captured by two onboard instruments: the Operational Land Imager (OLI) and the Thermal Infrared Sensor (TIRS) (Salakkham and Piyatadsananon, 2020). Based on the TIR bands of Landsat-8, the available data is appropriate to apply the split-window algorithms (SW) (Jimenez-Munoz et al., 2014) and three LST estimation methods of single-channel methods, Radiative Transfer Equation-based method (RTE), the Improved Mono-Window method (IMW), and the Generalized Single-Channel method (GSC) (Li et al., 2013). Weng et al. (2004) indicated that the emissivity estimation for ground objects from passive sensor data could be measured using different technics. Liu et al. (2006) and Coll et al. (2010) noted that LST estimation was performed over the large, fully vegetated surface, bare surfaces, and deserts, with relatively homogeneous test sites avoiding uncertainty due to spatial heterogeneity. Therefore, an optimal method to estimate the actual temperature from mixed land-use types in a pixel of Landsat-8 data has to be examined and criticized. This study aimed to explore the optimum method in LST estimation over four favoured algorithms, RTE, IMW, GSC, and SW, regarding their wide use in these recent years. As a result, the four LST retrieval algorithms were criticized and explored the limits of the applied parameters. This study benefits the GIS specialists working for local government to follow the best practice for the LST estimation over a city. Later, provincial officers can use the explored results from the optimal method to monitor the LST over a small and diverse city, such as Buriram Town Municipality.

MATERIALS AND METHODS

1. Land Surface Temperature (LST) estimation

Four LST (Land Surface Temperature) estimation algorithms have been typically employed in recent decades in response to remote sensing data. The RTE and IMW were developed by Wang et al. (2015). The GSC and SW were developed by Jimenez-Munoz et al. (2014). The first one the Radiative transfer equation-based method (RTE), was developed by Wang et al. (2015) with the concern of radiative transfer equation based on Plank's law inversion (Skokovic et al., 2014). The atmospheric profile was extracted from the NCEP (National Centers for Environmental Prediction) dataset and used to simulate atmospheric transmittance, up-welling, and down-welling radiance from the Moderate-resolution atmospheric Transmisi (MODTRAN) model (Salakkham and Piyatadsananon, 2020). The expresbrackets the RTE is,

$$LST = \frac{C_2}{\lambda\, ln\left\{\frac{C_1}{\lambda^5[\quad]} + 1\right\}} \tag{1}$$

Where L_{sensor} is thermal radiance at the sensor level, ε is land-surface emissivity, τ is atmospheric transmissivity, L_u and $\underline{L_d}$ are up-welling and down-welling atmospheric radiance, respectively, and C_1 and C_2 are the constant-coefficients.

Later, Qin et al. (2001) developed the mono-window algorithm. It was used for estimating the LST from LANDSAT-5 to avoid dependence on radio-sounding in the RTE method (Sobrino et al. 2004). Consequently, Wang et al. (2015) developed the mono-window into the Improved Mono-Window (IMW) method for obtaining LST from LANDSAT-8 in 2015 (Salakkham and Piyatadsananon, 2020), as shown in the following expression.

$$LST = \frac{1}{C}\left[a(1 - C - D) + \binom{b(1 - C - D) +}{C + D} T_B - DT_a\right] \tag{2}$$

With $C = \varepsilon\tau$

$D = (1 - \tau)[1 + (1 - \varepsilon)\tau]$

Where a and b are constant coefficients, ε is the land surface emissivity, τ is the total atmospheric transmissivity, T_B is the at-sensor brightness temperature, and T_a is the mean atmospheric temperature.

In 2003, Jimenez-Munoz and Sobrino (2003) initially developed the Generalized Single-Channel (GSC) algorithm to estimate the LST from LANDSAT-5. It was further developed in 2014 to obtain LST from LANDSAT-8, shown in the following expression Salakkham and Piyatadsnanon, 2020).

$$LST = \gamma[\varepsilon^1(\psi \;\; L_{sensor}\, \psi_2) + \psi_3] + \delta \tag{3}$$

$$\text{With } \gamma = \frac{T_B^2}{b_\gamma L_{sensor}} \tag{4}$$

$$\delta = T_B - \frac{T_B^2}{b_\gamma} \tag{5}$$

Where L_{sensor} is thermal radiance at the sensor level, b_γ equals 1,324 K, and 1,199 K for TIRS -1 (Band 10) and TIRS -2 (Band 11), respectively, T_B is at-sensor brightness temperature, ε is the land surface emissivity, and ψ_1, ψ_2, ψ_3 an be obtaned as a function of the total atmospheric water vapor content (w).

Eventually, the split-window algorithm, developed by Jimenez-Munoz et al. (2014), is shown in the following expression.

$$T_s = T_i + C_1(T_i - T_j) + C_2(T_i - T_j)^2 + C_0 + (C_3 + C_4 w)(1 - \varepsilon) + (C_5 + C_6 w)\Delta\varepsilon \tag{6}$$

Where C_0 to C_6 are the split window coefficients, T_i and T_j are at -sensor brightness temperatures of Band i and j, respectively, ε is the land surface emissivity obtained from $\varepsilon = 0.5\ (\varepsilon_i + \varepsilon j)$ and $\Delta\varepsilon = (\varepsilon_i - \varepsilon_j)$.

It is noted that the near-surface air temperature (T_0) and relative humidity were received from Huai Rat Station near Buriram Town Municipality (approximately twelve kilometers). As mentioned in a report by Salakkham and Piyatadsananon (2020) that near-surface air temperature (T_0) and relative humidity can be found on the Hydro and Agro Informatics Institute (HAII) website (Salakkham and Piyatadsananon, 2020). The parameters were used in the water vapor content calculation and estimation developed by Liu and Zhang (2011) as equation (7). The water vapor content has been used in the transmittance calculation for the IMW algorithm, the atmospheric function, the GSC algorithm, and the SW algorithm (Salakkham and Piyatadsananon, 2020). This parameter has also been used in the atmospheric temperature (T_a) calculation, an essential parameter of the IMW algorithm.

$$w_i = \left\{0.59 \times RH \times exp\left[\frac{17.27 \times (T_0-273.15)}{237.3+(T_0-273.15)}\right]\right\} + 0.1697 \quad (7)$$

Where w_i is the water vapor content (g cm^{-2}), T_0 is the near-surface air temperature (K), and RH is the relative humidity (Decimal). The water vapor content, near-surface air temperature, and relative humidity are the average values.

The transmittance, up-welling, and down-welling atmospheric radiance were obtained from the NASA atmospheric correction parameter calculator (Salakkham and Piyatadsananon, 2020). It is clearly shown that the calculator uses the National Center for Environmental Prediction (NCEP) to model global atmospheric profiles, which are interpolated to a particular date, time, and location as input for the MODTRAN radiative transfer code, and as a suite of the integrative algorithm to infer the up-welling, down-welling radiances and site-specific transmission (Salakkham and Piyatadsananon, 2020). The profiles resulting from time interpolation provide the closest latitudinal and longitudinal positions or specific locations (Vlassova et al., 2014).

2.Methods

2.1 Study Area

Buriram province is in the Northeastern region of Thailand. It has flourished over the last decade as a sports city in the country. A mega-sports complex, which contains a massive stadium for football, a motor racing track, and several ongoing construction projects, attracts many tourists and drives up the demand for further construction across Buriram Town Municipality (Buriram World, 2016; Tourism Authority of Thailand, 2017). The local government has planned to improve the Town Municipality to City Municipality to support urbanization (Buriram World, 2016). With six square kilometers for over 30,000 families living in the municipality, Buriram municipality is the most crowded city (around five-thousand population in a square kilometer) in the Northeastern region (The Bureau of Registration Administration, 2018). The maximum temperature in the summer has increased to more than 40 ºC (in 2013 – 2018) (Buriram Statistic Office, 2018).

2.2 Data collection

The data used in the LST estimation of this study are listed in table 1 with their sources.

Table 1. Data used in the study.

Data	Date	Sources
LANDSAT-8 data Path /Row: 128 /50	Jan 21st, Feb 6th, Mar 26th, Apr 11th, 2018	U.S. Geological Survey (USGS)
UAV image (GSD = 5cm.)	Mar-Apr, 2018	Surveying between 10-11 am.
Ground-based temperature data	Jan 21st, Feb 6th, Mar 26th, Apr 11th, 2018	Surveying between 10-11 am.
Atmospheric parameters • Air temperature • Relative humidity	Jan 21st, Feb 6th, Mar 26th, Apr 11th, 2018 Between 10-11 am.	Hydro and Agro Informatics Institute (HAII) website
Atmospheric parameters • Transmittance • Up-welling and down-welling atmospheric radiance	Jan 21st, Feb 6th, Mar 26th, Apr 11th, 2018	NASA atmospheric correction parameter calculator website

The atmospheric parameters used in the LST estimation of four methods are listed in table 2.

Table 2. Atmospheric parameters used in LST estimations.

		Jan 21st	Feb 6th	Mar 26th	Apr 11th
Temperature (T_0) (K)		303.9	293.1	302.9	309.3
Air Temperature (T_0) (ºC)		30.75	19.95	29.75	36.15
Relative Humidity		0.63	0.60	0.65	0.44
Water Vapor Content		2.86	1.52	2.79	2.72
Methods	**Atmospheric Parameters**	**Jan 21st**	**Feb 6th**	**Mar 26th**	**Apr 11th**
RTE	Transmittance (τ) [a]	0.53	0.80	0.54	0.60
	Up-welling	3.92	1.63	3.78	3.56
	Down-welling	6.00	2.67	5.86	5.65
IMW	Atmospheric Temperature (T_a)(K)	296.69	286.79	295.78	301.65
	Transmittance (τ) [b]	0.65	0.80	0.65	0.65
GSC	Atmospheric Function (ψ_1)	1.42	1.15	1.41	1.39
	Atmospheric Function (ψ_2)	-7.25	-2.97	-6.99	-6.70
	Atmospheric Function (ψ_3)	3.69	1.81	3.60	3.49
SW	Water Vapor Content	2.86	1.52	2.79	2.72

Note: [a] Transmittance, up-welling, and down-welling used in the RTE method were obtained from NCEP
[b] The transmittance used in IMW was calculated based on the mono-window method.

2.3 Procedures

The conceptual procedure of this study is illustrated in figure 2. It consists of three major parts, (1) ground-based temperature measurement, (2) LST estimation, and (3) the comparison between ground-based temperature data and the calculated LST data from the Landsat 8 data. Eventually, the optimum method of LST estimation was then identified by considering the lowest calculated NRMSE values.

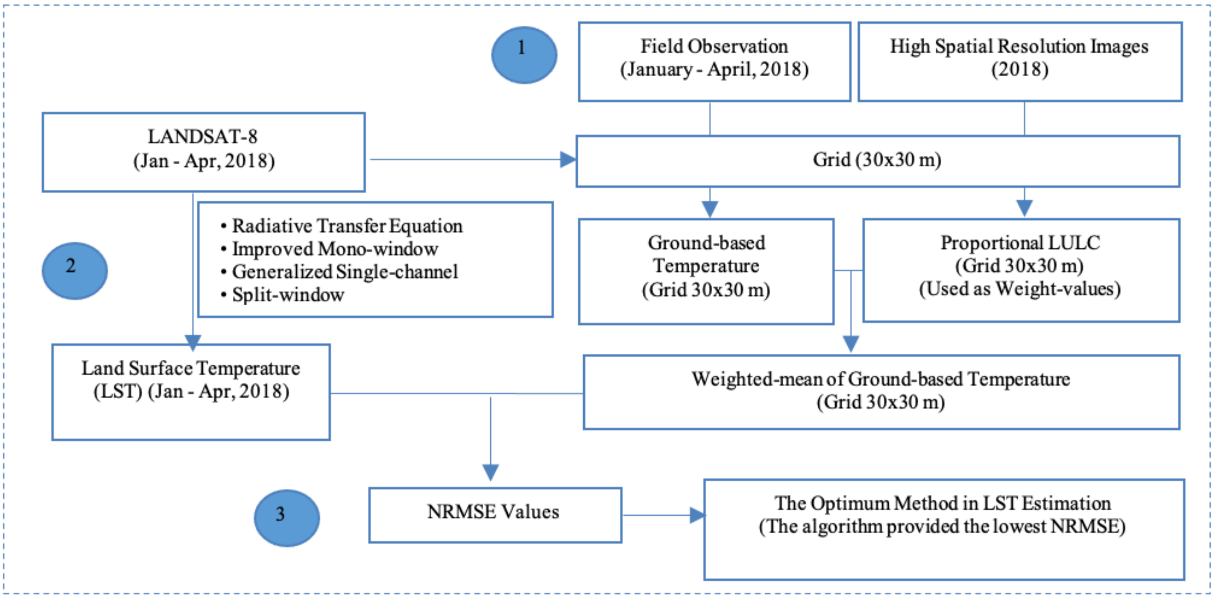

Figure 1. The conceptual procedure of the study.

2.4 Ground-based temperature measurement

Basically, the LANDSAT-8 image contains 30x30 meter-grids, whereas the thermal band image consists of 100x100 meter-grids. Therefore, an aggregated pixel of 3x3 pixels (90x90 m.) of OLI was assembled to be a pixel size of the Thermal image (100x100 m.). The Land-Use and Land-Cover (LULC) types within a thermal pixel (100x100 m.) were classified by visual interpretation technic on the high-resolution image from the UAV. The LULC within a thermal grid cell was assessed as the weighted value regarding the proportion of the mixed LULC. The high-resolution image from the UAV was also used as the based map for planning the ground-based temperature measurement. A hand-held digital thermometer was calibrated every time before measuring the LST in the sample sites. One hundred sample sites were measured and recorded the land surface temperature in the center of the 3x3 pixels of OLI, or 90x90 m. of thermal bands during 10-11 am. on Jan 21st, Feb 6th, Mar 26th, and Apr 11th, 2018.

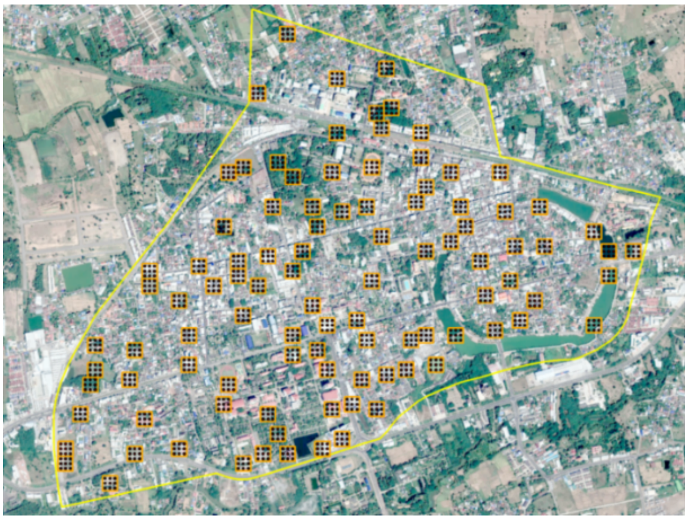

Figure 2. The 100 sample sites (small orange grids) over Buriram Municipality (yellow boundaries).

2.5 Accuracy assessment

The optimum method in the LST extraction, RTE, IMW, GSC, and SW algorithm, as equation (1) – (7), were used in the calculations. Eventually, the method that provides the lowest NRMSE values is considered an optimum method for the LST estimation of Buriram Town Municipality.

$$NRMSE = \frac{RMSE}{maximum\ observation - minimm\ observation} \quad (8)$$

Where maximum and minimum observations are the maximum and minimum temperature of in-situ data.

RESULTS AND DISCUSSION

The result from the split-window technique shows a more complex surface than others (Figure 3). As a reason, two thermal infrared channels have narrower bandwidths, which can capture finer land surface information response to studies by Li, et al. (2013) (Wenbin et al., 2013; Du et al., 2015). The accuracy assessment was examined to explore the optimum method in the LST estimation by considering the lowest NRMSE value of each method. The RMSE and NRMSE values as shown in table 3 and figure 4. The SW method eliminates atmospheric effects based on differential absorption in two thermal bands with narrow bandwidths in the thermal infrared (Salakkham and Piyatadsananon, 2020). As supported by the studies of Caselles et al. (1998) and Rozenstein et al. (2014), whether two separating narrow thermal infrared presents the smallest error in the LST retrieval. However, the SW algorithm is sensitive to water vapor content and coefficients. Typically, the coefficients used in the SW algorithm are based on the series of studies (Jimenez-Munoz and Sobrino, 2008; Jimenez-Munoz et al. 2014; Jin et al., 2015). In addition, the coefficients depend on the atmospheric state, while the fixed values were sometimes utilized, causing significant errors to the results, as highlighted in a study by Vazquez et al. (1997).

On the other hand, the single-channel methods, RTE, IMW, and GSC algorithms, rely on the accuracy of the radiative transfer model and the atmospheric profiles representing the actual state of the atmosphere over the studies area at the orbital time (Salakkham and Piyatadsananon, 2020). The error of the RTE algorithm comes from the atmospheric model used in the calculation of the atmospheric parameters. Since the study area is located in the tropical zone, the available model is the NCEP model presenting the mid-latitude summer and mid-latitude winter models, as same as the discussion in a study by Jimenez-Munoz et al. (2009). The error from the IMW algorithm comes from the essential atmospheric parameters used in this algorithm. There is no reference source in near-ground air temperature (T_0) acquisition, used in the sufficient atmospheric temperature (T_a). The sufficient atmospheric temperature (T_a) is an essential practical issue used to retrieve LST over a large area, as emphasized by Cristobal et al. (Cristobal et al. 2009). Lastly, the GSC algorithm provides a higher error than other methods. The basis of this algorithm relies on the estimation of the so-called atmospheric function, which is assumed to be dependent only on water vapor content values (Salakkham and Piyatadsananon, 2020). Chen et al. (2011) and Cristobal et al. (2009) explained that the atmospheric functions might be obtained more precisely from water vapor content and air temperature.

Table 3. NRMSE Values of the LST estimation methods

Date	NRMSE			
	RTE	IMW	GSC	SW
Jan 21st, 2018	0.454	0.613	0.650	0.227
Feb 6th, 2018	0.702	0.317	0.671	0.473
Mar 26th, 2018	0.246	0.416	0.509	0.218
Apr 11th, 2018	0.165	0.226	0.202	0.132
Overall NRMSE	0.171	0.181	0.219	0.114

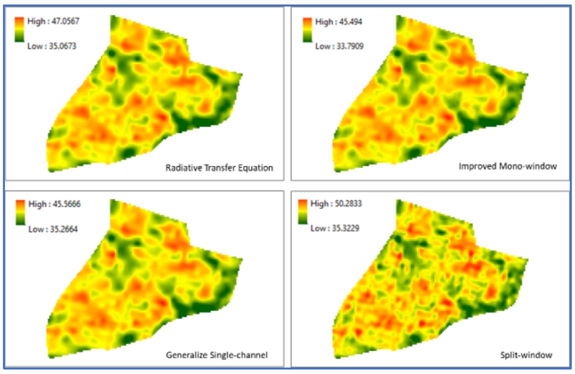

Figure 3. LST calculated by four methods.

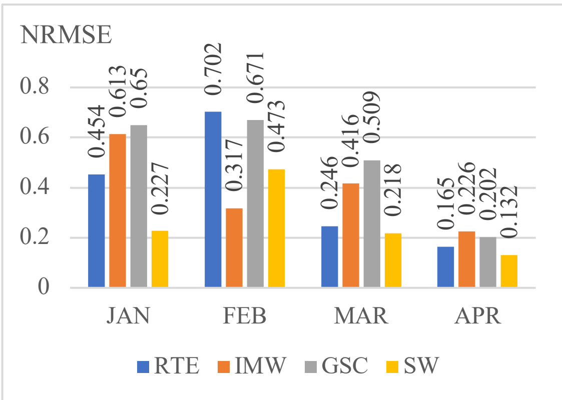

Figure 4. NRMSE values, monthly results.

CONCLUSIONS

It can be concluded that the SW algorithm provided the lowest NRMSE value (0.114); nevertheless, the IMW algorithm provided a better result than the SW algorithm on Feb 6th, 2018. It is noticeable that the average temperature on Feb 6th, 2018 was, dramatically dropped to 19.95 ºC, shown in table 2, so it caused the lowest water vapor content

(1.52 g cm^{-2}) in Feb 2018. For this reason, the critical point is that the amount of atmospheric water vapor content data plays an essential role in calculating accuracy. This parameter is typically estimated by considering the near-surface air temperature and relative humidity values (Salakkham and Piyatadsananon, 2020). To enhance the accuracy of all practiced algorithms, it is recommended to obtain the near-surface air temperature and relative humidity in-situ of the study area. Apart from the atmospheric correction parameters, the surface emissivity must also be considered to enhance the accuracy of the LST retrieval. The study area, Buriram Town Municipality, appears as heterogeneous LULC within a thermal-image pixel, causing different spectrum reflectance. Regarding coarse spatial resolution, it is strongly affected by mixed pixels, whereby each pixel comprises a mixture of two or more land cover types (Feng et al., 2015). Therefore, the SW algorithm is recommended for the LST estimation in a small city like Buriram municipality. It can be done regularly using the Landsat-8 data with the sub-pixel technique, which can monitor the LST in small cities. Considering the limitation of the parameters used in the SW method, it is suggested that a local weather station in the city should be identified to provide the near-surface air temperature and relative humidity values. The explored optimum method for the LST estimation from this study will be useful to GIS specialists who are working for the local government to conduct this technic to prevent the urban heat island (UHI) in small cities with similar contents.

REFERENCES

Avdan, U., and Jovanovska, G. 2016. Algorithm for automated mapping of the land surface temperature using Landsat-8 satellite data. Journal of Sensors. 2016: 1-8.

Bendib, A., Dridi, H., and Kalla, M. I. 2016. Contribution of Landsat-8 data for the estimation of land surface temperature in Batna City, Eastern Algeria. Geocarto International. https://doi.org/10.1080/10106049.2016.1156167

Buriram Statistic Office. 2018. Monthly temperature and atmospheric pressure data 2013-2018. Retrieved from http://buriram.old.nso.go.th/nso/project/search_option/search_result.jsp

Buriram World. 2016. Retrieved from https://www.buriramworld.com/

Caselles, V., Rubio, E., Coll, C., and Valor, E. 1998. Thermal band selection for the PRISM instrument 3: Optimal band configurations. Journal of Geophysical Research. 103: 17057-17067.

Chen, F., Zhao, X., Ye, H., and Hu, H. 2011. Retrieving land surface temperature from Landsat TM using different atmospheric products as ancillary data. Paper presented at the Spatial Data Mining and Geographic Knowledge Services (ICSDM), Fuzhou, China.

Coll, C., Galve, J. M., Sanchez, J. M., and Caselles, V. 2010. Validation of Landsat-7/ETM+ thermal-band calibration and atmospheric correction with ground-based measurements. IEEE Transactions on Geoscience and Remote Sensing. 48(1): 547-555.

Cristobal, J., Jimenez-Munoz, J. C., Sobrino, J. A., Ninyerola, M., and Pons, X. 2009. Improvements in land surface temperature retrieval from the Landsat series thermal band using water vapor and air temperature. Journal of Geophysical Research. 114. https://doi.org/10.1029/2008JD010616

Du, C., Ren, H., Qin, Q., Meng, J., and Zhao, S. 2015. A practical split-window algorithm for estimating land surface temperature from Landsat-8 data. Remote Sensing. 7: 647-665.

Feng, X., Foody, G., Aplin, P., and Gosling, S. N. 2015. Enhancing the spatial resolution of satellite-derived land surface temperature mapping for urban areas. Sustainable Cities and Society. 19: 341-348.

Holmes, T. R. H., Crow, W. T., Yilmaz, M. T., Jackson, T. J., and Basara, J. B. 2013. Enhancing model-based land surface temperature estimates using multiplatform microwave observations. Journal of Geophysical Research: Atmospheres, 118: 577-591.

Jimenez-Munoz, J. C., Cristobal, J., Sobrino, J. A., Soria, G., Ninyerola, M., and Pons, X. 2009. Revision of the single-channel algorithm for land surface temperature retrieval from Landsat thermal-infrared data. IEEE Transactions on Geoscience and Remote Sensing. 47(1): 339-349.

Jimenez-Munoz, J. C., and Sobrino, J. A. 2003. A Generalized single-channel method for retrieving land surface temperature from remote sensing data. Journal of Geophysical Research. 108: 1-9.

Jimenez-Munoz, J. C., and Sobrino, J. A. 2008. Split-window coefficients for land surface temperature retrieval from low-resolution thermal infrared sensors. IEEE Geoscience and Remote Sensing Letters. 5(4): 806-809.

Jimenez-Munoz, J. C., Sobrino, J. A., Skokovic, D., Matter, C., and Cristobal, J. 2014. Land surface temperatures retrieval methods from Landsat-8 thermal infrared sensor data. IEEE Geoscience and Remote Sensing Letters. 11(10): 1840-1843.

Jin, M., Li, J., Wang, C., and Shang, R. 2015. A practical split-window algorithm for retrieving land surface temperature from Landsat-8 data and a case study of an urban area in China. Remote Sensing. 7: 4371-4390.

Li, Z. L., Tang, B. H., Wu, H., Ren, H., Yan, G., Wan, Z., and Sobrino, J. A. 2013. Satellite-derived land surface temperature: Current status and perspective. Remote Sensing of Environment. 131: 14-37.

Liu, L., and Zhang, Y. 2011. Urban heat island analysis using the Landsat TM data and ASTER data: A case study in Hong Kong. Remote Sensing. 3: 1535-1552.

Liu, Y., Hiyama, T., and Yamaguchi, Y. 2006. Scaling of land surface temperature using satellite data: A case examination on ASTER and MODIS products over a heterogeneous Terrain Area. Remote Sensing of Environment. 105: 115-128.

Mechri, R., Ottle, C., Pannekoucke, O., and Kallel, A. 2014. Genetic particle filter application to land surface temperature downscaling. Journal of Geophysical Research: Atmospheres. 119: 2131-2146.

Orhan, O., Ekercin, S., and Dadaser-Celik, F. 2014. Use of Landstat land surface temperature and vegetation indices for monitoring drought in the Salt Lake Basin Area, Turkey. The Scientific World Journal. 2014: 1-11.

Qin, Z., Karnieli, A., and Berliner, P. 2001. A mono-window algorithm for retrieving land surface temperature from Landsat TM data and its application to the Israel-Egypt border region. International Journal of Remote Sensing. 22(18): 3719-3746.

Rozenstein, O., Qin, Z., Derimian, Y., and Karnieli, A. 2014. Derivation of land surface temperature for Landsat-8 TIRS using a split-window algorithm. Sensors. 14: 5768-5780.

Salakkham, E., and Piyatadsananon, P. 2020. The optimum method for urban land surface temperature estimation. preprints 2020. Seminar Report. Suranaree University of Technology.

Skokovic, D., Sobrino, J. A., Jimenez-Munoz, J. C., Soria, G., Julien, Y., Matter, C., and Cristobal, J. 2014. Calibration and validation of land surface temperature for Landsat-8 TIRS sensor. Retrieved from https://earth.esa.int/documents/700255/2126408/ESA_Lpve_Sobrino_2014a.pdf

Sobrino, J. A., Jimenez-Munoz, J. C., and Paolini, L. 2004. Land surface temperature retrieval from Landsat TM 5. Remote Sensing of Environment. 90: 434-440.

Tourism Authority of Thailand. 2017. Buriram. Retrieved from https://www.tourismthailand.org/About-Thailand/Destination/Buri-Ram

Vazquez, D. P., Reyes, F. J. O., and Arboledas, L. A. 1997. A comparative study of algorithms for estimating land surface temperature from AVHRR data. Remote Sensing of Environment. 62: 215-222.

Vlassova, L., Perez-Cabello, F., Nieto, H., Martin, P., Riano, D., and Riva, J. d. l. 2014. Assessment of methods for land surface temperature retrieval from Landsat-5 TM images applicable to multiscale tree-grass ecosystem modeling. Remote Sensing. 6: 4345-4368.

Wang, F., Qin, Z., Song, C., Tu, L., Karnieli, A., and Zhao, S. 2015. An improved mono-window algorithm for land surface temperature retrieval from Landsat-8 thermal infrared sensor data. Remote Sensing. 7: 4268-4289.

Wenbin, L., Yonghua, S., Dan, M., and Xiaojuan, L. 2013. Analysis of Beijing urban heat island under the influence of extreme heat based on HJ-1B data. Paper presented at the 2013 IEEE International Geoscience and Remote Sensing Symposium.

Weng, Q., and Fu, P. 2014. Modeling annual parameters of cleat-sky land surface temperature variations and evaluating the impact of cloud cover using time series of Landsat TIR Data. Remote Sensing of Environment. 140: 267-278.

Weng, Q., Fu, P., and Gao, F. 2014. Generating daily land surface temperature at Landsat resolution by fusing Landsat and Modis data. Remote Sensing of Environment. 145: 55-67.

Weng, Q., Lu, D., and Schubring, J. 2004. Estimation of land surface temperature-vegetation abundance relationship for urban heat island studies. Remote Sensing of Environment. 89: 467-483.

Wu, P., Shen, H., Ai, T., and Liu, Y. 2013. Land surface temperature retrieval at high spatial and temporal resolutions based on multi-sensor fusion. International Journal of Digital Earth. 6(1), 113-133.

Wu, P., Shen, H., Zhang, L., and Gottsche, F.M. 2015. Integrated fusion of multi-scale polar-orbiting and geostationary satellite observations for the mapping of high spatial and temporal resolution land surface temperature. Remote Sensing of Environment. 156, 169-181.

Zhou, J., Dai, F., Zhang, X., Zhao, S., and Li, M. 2015. Developing a temporally land cover-based look-up table (TL-LUT) method for estimating land surface temperature based on AMSE-E data over the Chinese landmss. International Journal of Applied Earth Observation and Geoinformation. 34: 35-50.

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Effect of N_2-fixing and IAA synthesis endophytic bacteria on growth of *Vanda* under greenhouse condition

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ABSTRACT

Vanda is one of the orchid genera that are important commercial crops. It was traded in both cut flower and plant form. In 2018, the *Vanda* export of Thailand had a marketable value of about 0.13% as cut flowers and 8.9% as orchid plants. Orchid cultivation is usually used a large number of fertilizers for growth and development, which lead to high cost and environmental problems. Therefore, this research was aimed to study N_2-fixing and IAA produced by endophytic bacteria as biofertilizers under greenhouse conditions. This research was conducted in a completely randomized design (CRD), and *Vanda* plantlets from tissue culture were inoculated with isolated 3S19 endophytic bacteria compared with deionized water (as a control treatment). Each treatment had twenty replications. The results showed that there were no significantly different between isolate 3S19 inoculation plants and with sterilized deionized water in plant height and leave number at 2, 3, and 4 months after inoculation. In contrast, root length and the number of roots were significantly different at 4 months after inoculation. The data from this research is beneficial as primary data for biofertilizer application to *Vanda* orchid.

Keywords: *Vanda*, orchid, endophytic bacteria, biofertilizer

INTRODUCTION

Orchids are an important commercial crop as an ornamental plant, potted plant, medical material, and industrial material (Hinsley et al., 2018). In 2018, Thailand exported cut flower and orchid plants for about 6.81 million US dollars (Department of International Trade Promotion, 2020). *Vanda* is one of the orchid exporting products with a market value of about 0.13% as cut flower form and 8.9% as an orchid plant (De et al., 2015; Panjama, 2018). Orchid cultivation has many factors to promote quality and yield, such as water, fertilizer, greenhouse, microorganism, etc. Generally, Thai farmers use a large number of fertilizers as water-soluble fertilizer forms, i.e. 20-20-20 or 21-21-21 (balance fertilizer) for the vegetative stage, 10-52-17 during floral stimulation, and 16-21-27 for growth and development (Department of Agriculture, 2017). The above information indicated that Thai growers used large amounts of fertilizers, resulting in high costs for orchid production. In addition, using chemical fertilizer leads to chemical accumulation in the environment because chemical fertilizer is slowly disintegrating (Ray et al., 2018. Micro-organisms, including bacteria or fungi, are one of the alternative products to promote plant growth through organic matter synthesis and are eco- friendly to the environment.

Endophytic bacteria is one micro-organism that lives inside the tissue of the plant and does not cause plant disease to the host plant (Lacava and Azevedo, 2013). It can promote host plant growth and development through many mechanisms such as plant growth promoter substance synthesis, e.g., Indole-3-acetic acid (IAA), gibberellic acid, and cytokinin substance, promoting nutrient or water absorbance, nitrogen fixation, and biological control (Lacava and Azevedo, 2013).

Therefore, this research was aimed to study N_2-fixing and IAA synthesis by endophytic bacteria to promote *Vanda* growth under greenhouse conditions. This basic information may be useful for biofertilizer production in the future.

MATERIALS AND METHODS

This research was conducted with two treatments. *Vanda* plants were inoculated with isolate 3S19 at a ratio of 1: 1 (endophytic bacterial suspension: sterilized water) compared with deionized water (as control treatment). There were 20 replications per treatment.

Preparing of endophytic bacteria suspension

The isolate 3S19 of endophytic bacteria, isolated from *Vanda* 'Manuvadee'which has the capability to fix nitrogen and synthesize IAA. (Inkaewpuangkham et al., 2021a; Inkaewpuangkham et al., 2021b). The assessment of nitrogen-fixing was conducted according to Weaver and Danso (Weaver and Danso, 1994), by acetylene reduction assay and an IAA synthesis estimating according to a modified version of the Gordon and Weber method (Gordon and Weber, 1951). Firstly, the isolate 3S19 was cultured in a nutrient agar (NA) medium for seven days. Then, a single colony of isolate 3S19 was cultured in nutrient broth (NB) medium at 25 C˚ and shaken at 120 rpm for three days. Finally, endophytic bacteria suspension was estimated at an optical density of 0.5 at 600 nm (Lertjantarangkool et al., 2017) (approximately 10^8 cfu/ml) before use.

Inoculation in Vanda plantlet

Vanda hybrid 6 months old plantlets from tissue culture were immersed in isolate 3S19 endophytic bacteria suspension compared with sterilized deionized water for 30 minutes. Then, inoculated plantlets were transferred to the basket and cultivated under greenhouse conditions for four months. The average temperature in the greenhouse was about 28˚C and 70% RH.

Data collection

The data collection were plant height, number of leaves, root length, and number of roots at 2, 3, and 4 months after inoculation. The data was collected once times per month.

Statistical analyses

The data were analyzed by T-test independent using the Sxw program (Analytical Software version 8.0) with a significant difference at 95% probability level.

RESULTS

Growth of plantlet

The height of inoculated plantlets was not significantly different from control treatments at 2, 3, and 4 months after inoculation (Table 1).

Table 1. Height (cm.) of *Vanda* at 2, 3, and 4 months after inoculation with bacterial endophytes or sterilized deionized water (control).

Treatments	**Months after inoculation (month)**		
	2	**3**	**4**
Inoculated *Vanda*	8.3	8.5	8.6
Control plant	7.9	8.1	8.3
T-test	ns	ns	ns

ns = not significant

The number of leaves per plant of *Vanda* increased 4 months after inoculation. However, there was no significantly different between treatments at 2, 3, and 4 months (Table 2).

Table 2. Number of leaves of *Vanda* at 2, 3, and 4 months after inoculation with bacterial endophytes or sterilized deionized water (control)

Treatments	**Months after inoculation (month)**		
	2	**3**	**4**
Inoculated *Vanda*	4.2	4.5	5.1
Control plant	4.2	4.7	5.3
T-test	ns	ns	ns

ns = not significant

There was no significantly different in root length of *Vanda* between treatments at 2 and 3 months after inoculation. However, there was a significantly different root length between treatments at 4 months. The inoculation treatment with isolate 3S19 showed higher root length than the control plant (Table 3.).

Table 3. Root length (cm.) of *Vanda* at 2, 3, and 4 months after inoculation with bacterial endophytes or sterilized deionized water (control)

Treatments	Months after inoculation (month)		
	2	3	4
Inoculated *Vanda*	1.1	2.0	2.9^a
Control plant	1.0	1.8	2.2^b
T-test	ns	ns	*

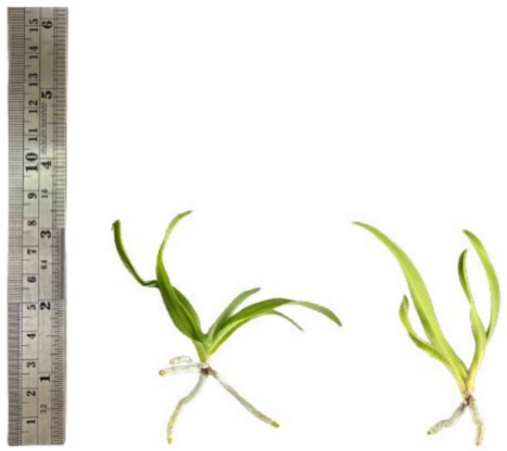
Data analysis by T-test
ns = not significant
* = significant difference at 95% ($P< 0.05$)

At 2 and 3 months after inoculation, there was no significantly different between treatments. Nevertheless, inoculated *Vanda* with isolate 3S19 had more roots than control plants at 3.8 and 3.3 roots per plant, respectively (Table 4.).

Table 4. Number of roots of *Vanda* at 2, 3, and 4 months after inoculation with bacterial endophytes or sterilized deionized water

Treatments	Months after inoculation (month)		
	2	3	4
Inoculated *Vanda*	2.8	3.5	3.8^a
Control plant	2.9	3.2	3.3^b
T-test	ns	ns	*

Data analysis by T-test
ns = not significant
* = significant difference at 95% ($P< 0.05$)



A B

Figure 1. Growth of *Vanda* plantlet after 4 months inoculation with isolate 3S19 (A), with sterilized deionized water (control) (B).

DISCUSSION

Plant height and leaf number of *Vanda* plantlet during 2-3 months after inoculation were not significantly different. It might be associated with host plant and bacteria interaction. The success of plant-bacteria associate depends on several factors. The condition of the environment is one factor for endophytic bacteria colonization, including season, temperature, latitude, longitude, nutrient, soil conditions, and so on (Chiellini et al., 2014; Yang et al., 2017; Wu et al., 2021). For example, the study of Ou et al. (2019) revealed that in the spring season, Actinobacteria and Proteobacteria of endophytic bacteria were found to be the most abundant in mulberry (*Morus* L.), but in the fall season found only Proteobacteria. In addition, the host plant is the main factor for endophytic bacteria colonization, consists of the plant growth stage, plant physiology, plant tissue type: Santos et al. (2018) showed that the plant tissue type leads to the different community of endophytic bacteria in host plant, plant species that similar Engelhard et al. (2000) reported that wild rice species and traditional race rice had a population of *Azoarcus* sp. higher than modern varieties of rice since the influence of plant species. Likewise, the plant defense process is associated with plant-bacteria interaction: in some genera bacteria can induce plant defensive process that results in colonization difficult by other endophytic bacteria into the host plant (Santos et al., 2018). Furthermore, micro-organism species are an important factor for entry to plants. Research by Nogueira et al. (2001) reported that plant responses involve in plant-gene expression which was found associated with bacteria and a specific bacterial species.

Root length and number of roots of inoculated *Vanda* at 4 months after inoculation showed better growth than the control treatment. It might be because of abundant endophycic bacteria in the root organ. Typically, endophycic bacteria start entering into host plant via the roots because the root plant produces exudates to interact with bacteria. After endophytic bacteria entry to the root, they can spread to colonize above ground tissue by the movement of bacteria (bacterial flagella) and plant transpiration stream (Afzal et al., 2019). So, the 3S19 endophytic bacteria might be more abundant in roots than in other organs resulting in the root zone had more IAA synthesis by endophytic bacteria than the above zone. The property of isolate 3S19 could produce indole-3-acetic acid (IAA) without and with L-tryptophan medium at 9.48 and 17.38 mg IAA/l, respectively (Inkaewpuangkham et al., 2021a). Indicating that this isolate was active to produce IAA without exogenous tryptophan. Similarly, the previous report found that orchid roots are associated with fungal and bacterial symbionts, and they can synthesize IAA into orchids (Novak et al., 2014). Furthermore, a similar result was revealed by Khalid et al. (2004) that microorganisms isolated from plant rhizoferic soil could synthesize IAA by using exudate containing tryptophan substrate from the plant root released. Since IAA is a plant growth regulator classified in the auxin group, it is predominant for promoting root formation or root initiation and enhancing root growth (Novak et al., 2014). Thus, isolating 3S19 could effectively promote root growth. Similar Tsavkelova et al. (2007) research reported that bacteria were isolated from root terrestrial orchids and epiphytic tropical orchids. These bacteria can produce IAA and stimulate root formation of kidney bean cutting.

The above reasons indicate that most bacteria often enter through roots and spread to the above plant part, and isolate 3S19 might have an intensive bacteria population in roots than leaves. So, isolate 3S19 might affect root growth than leave growth in this stage of *Vanda*.

CONCLUSIONS

In conclusion, *Vanda* plantlets inoculated with isolate 3S19 of endophytic bacteria at a ratio of 1:1 (endophytic bacterial suspension: sterilized water) affected the growth of *Vanda* roots in both root length and the number of roots per plant than with sterilized deionized water inoculation. However, the data from this research is basic information to develop into a biofertilizer product. In the next step, the researcher will assess aspects of this isolate, such as phosphate solubilization and siderophore synthesis.

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REFERENCES

Afzal, I., Shinwari, Z.K., Sikandar, S., and Shahzad, S. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiological Research. 221: 36-49. https://doi.org/10.1016/j.micres.2019.02.001.

Chiellini, C., Maida, I., Emiliani, G., Mengoni, A., Mocali, S., Fabiani, A., Biffi, S., Maggini, V., Gori, L., Vannacci, A., Gallo, E., Firenzuoli, F., and Fani, R. 2014. Endophytic and rhizospheric bacterial communities isolated from the medicinal plants *Echinacea purpurea* and *Echinacea angustifolia.* International Microbiology. 17: 165-174. https://doi.org/10.2436/20.1501.01.219.

De, L., Pathak, P., Rao, A.N., and Rajeevan, P.K. 2015. Commercial Orchids. De Gruyter Open Ltd, Berlin.

Department of Agriculture. 2017. Orchid Production Technology. Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok.

Department of International Trade Promotion. 2020. Orchid Products. Department of International Trade Promotion, Ministry of Commerce, Thailand.

Engelhard, M., Hurek, T., and Reinhold-Hurek, B. 2000. Preferential occurrence of diazotrophic endophytes, *Azoarcus* spp., in wild rice species and land races of Oryza sativa in comparison with modern races. Environmental Microbiology. 2(2): 131-141. https://doi.org/10.1046/j.1462-2920.2000.00078.x

Gordon, S. A., and Weber, R. P. 1951. The colorimetric estimation of indole acetic acid. Plant Physiology. 26: 192-195. https://doi.org/10.1104/pp.26.1.192.

Hinsley, A., Boer, H.J.DE., Fay, M.F., Gale, S.W., Gardiner, L.M., Gunasekara, R.S., Kumar, P., Masters, S., Metusala, D., Roberts, D. L., Veldman, S., Wong, S., and Phelps, J. 2018. A review of the trade in orchids and its implications for conservation**.** Botanical Journal of the Linnean Society. 186: 435-455. https://doi.org/10.1093/botlinnean/box083**.**

Inkaewpuangkham, W., Panjama, K., Inkham, C., Chromkaew, Y., and Ruamrungsri, S. 2021a. Isolation and Selection Potential N-Fixing of Endophytic Bacteria in *Vanda* Orchid. In: Sum-im, T. (ed) The 14th Research Administration Network Conference 2021 (online). Bangkok, 5-6 Aug 2021. Strategic Wisdom and Research Institute, Srinakharinwirot University. p.87-95.

Inkaewpuangkham, W., Panjama, K., Inkham, C., Chromkaew, Y., and Ruamrungsri, S. 2021b. Assessment of IAA synthesis by endophytic bacteria in *Vanda* (Orchidaceae) (Book of Abstracts). In: Thammasiri, K., Kongsawadworakul, P., and Swangpol, S.C. (eds) The IX International Scientific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation (Physiological, Biochemical, Embryological, Genetic and Legal Aspects) (Biotech 2021). Faculty of Science, Mahidol University, Bangkok, Thailand, 12-13 Jul 2021. Department of Plant Science, Faculty of Science, Mahidol University. p.31.

Khalid, A., Arshad, M., and Zahir, Z.A. 2004. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. Journal of applied microbiology. 96: 473-480. https://doi.org/10.1046/j.1365-2672.2003.02161.x.

Lacava, P.T., and Azevedo, J.L. 2013. Chapter 1 Endophytic Bacteria: A Biotechnological Potential in Agrobiology System. In: Maheshwari, D.K., Saraf, M., and Aeron, A. (ed) Bacteria in Agrobiology: Crop Productivity. Springer, New York. p.1-44.

Lertjantarangkool, T., Pengnoo, A., Punsak, W., and Homhaul, W. 2017. Effectiveness of free living nitrogen fixing bacteria on seed germination of rice. In: Wattanachaiyingcharoen, D., et al. (eds) Naresurn research conference 13. Phitsanulok, 20-21 July 2017. Naresurn University. P.359-367.

Nogueira, E.M., Vinagre, F., Masuda, H.P., Vargas, C., Muniz de Pádua, V.L., Da Silva, F.R., Dos Santos, R.V., Baldani, J.I., Ferreira, P.C.G., and Hemerly, A.S. 2001. Expression of sugarcane genes induced by inoculation with *Gluconacetobacter diazotrophicus* and *Herbaspirillum rubrisubalbicans*. Genetics and Molecular Biology. 24: 199-206. https://doi.org/10.1590/S1415-47572001000100027.

Novak, S.D., Luna, L.J., and Gamage, R.N. 2014. Role of Auxin in Orchid Development. Plant Signaling & Behavior. 9(10): e972277-1- e972277-8. https://doi.org/10.4161/psb.32169.

Ou, T., Xu, W., Wang, F., Strobel, G., Zhou, Z., Xiang, Z., Liu, J., and Xie, J. 2019. A microbiome study reveals seasonal variation in endophytic bacteria among different Mulberry cultivars. Computational and Structural Biotechnology Journal. 17: 1091–1100. https://doi.org/10.1016/j.csbj.2019.07.018.

Panjama, K. 2018. Nitrogen uptake and assimilation in *Vanda* hybrid. Ph.D. Dissertation. Graduate school Chiang Mai University, Chiang Mai, Thailand.

Ray, S., Singh, J., Rajput, R.S., Singh, H.B., and Singh, S. 2018. Endophytic Bacteria: an Essential Requirement of Phyto Nutrition. Nutrition and Food Science International Journal. (5)2: 1-5. https://doi: 10.19080/NFSIJ.2018.05.555657.

Santos, M.L.dos., Berlitz, D.L., Wiest, S.L.F., Schünemann, R., Knaak, N., and Fiuza, L.M. 2018. Benefits Associated with the Interaction of Endophytic Bacteria and Plants. Brazilian Archives of Biology and Technology. 61: e18160431, 1-10. https://doi.org/10.1590/1678-4324-2018160431.

Tsavkelova, E.A., Cherdyntseva, T.A., Botina, S.G., and Netrusov, A.I. 2007. Bacteria associated with orchid roots and microbial production of auxin. Microbiological Research. 162: 69-76. https://doi.org/10.1016/j.micres.2006.07.014.

Weaver, R. W., and Danso, Seth K. A. 1994. Dinitrogen fixation. In: Weaver, R.W., Angle, J.S., and Bottomley, P.S. (eds.) Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties. Soil Science Society of America, Inc., Madison. p. 1019-1043.

Wu, W., Chen, W., Liu, Shiyu., Wu, Jianjun., Zhu, Yeting., Qin, Luping., and Zhu, Bo. 2021. Beneficial Relationships Between Endophytic Bacteria and Medical Plants. Frontiers in Plant Science. 12: 1-13. https://doi.org/10.3389/fpls.2021.646146.

Yang, R., Liu, P., and Ye, W. 2017. Illumina-based analysis of endophytic bacterial diversity of tree peony (*Paeonia* Sect. *Moutan*) roots and leaves. Brazilian Journal of Microbiology. 48: 695-705. https://doi.org/10.1016/j.bjm.2017.02.009.

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Comparison of hemp (*Cannabis sativa* L.) seed oil by conventional and soxhlet extraction methods

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ABSTRACT

Hemp (*Cannabis sativa* L.) is a multi-purpose plant that recently got lots of attention in Thailand. In previous years, various studies have been done regarding the application of hemp fiber, especially in the textile sector. After the government has unlocked regulation regarding hemp cultivation and its application, hemp seed has become a remarkable part as oil content in the seeds can be used for multiple purposes. The hemp oil extraction process becomes more important to get a higher yield. So, this research interest has been focused on comparing conventional and soxhlet extraction methods using water, n-hexane, and petroleum ether as extraction solvents. The experimental results showed that solvent has an impact on the extraction efficiency of hemp seed oil as well as the extraction method. Petroleum ether as a solvent by the soxhlet extraction method showed a significantly ($P<0.05$) higher yield (24.44%) compared to those of conventional extraction (17.06%). Additionally, using n-hexane as a solvent by the soxhlet extraction method showed a higher yield (21.65%) than the conventional extraction (18.78%). However, water has better efficiency in conventional extraction (4.56%) than the soxhlet extraction method (1.27%). According to the experimental results, the soxhlet extraction method using petroleum ether as an extraction solvent is recommended to get a higher oil yield from hemp seed.

Keywords: hemp seed, oil extraction method

INTRODUCTION

Hemp (*Cannabis sativa* L.) and marijuana were classified by morphological characteristics and phytochemistry. Hemp and marijuana are plants in temperate regions of Asia. In the past, both used to benefit fiber, and the cultivation of a plant used to make a popular local narcotic. This plant is widely known to be the major source of Cannabinoids such as Cannabidiol (CBD), Tetrahydrocannabinol (THC), and cannabinol (CBN). Cannabinoids have been shown vital addictive substances and affect the brain. Cannabidiol (CBD) has active anti-tetrahydrocannabinol, which effectively reduces anxiety symptoms and pain relief. (Pinmanee, 2019).

Currently, Thailand has liberalized the permission for hemp cultivation and its application in the food, cosmetic, and medical sectors. Availability of hemp in Thailand, the Highland Research and Development Institute (Public Organization) has been developed the hemp variety and promotes the cultivation of hemp as a new economic crop with the suitable and higher potential of 4 varieties (RF1-4) (Pinmanee, 2019).

Hemp seed oil is valued primarily for its nutritional properties and the health benefits associated with it. Although its fatty acid composition is most often noted, with oil content ranging from 25-35%, whole hemp seed is additionally comprised of approximately 20-25% protein, 20-30% carbohydrates, 10-15% fiber, and 25% fat along with an array of trace minerals (Deferne and Pate, 1996). Fat consists of essential fatty acids or omega 6 vs. omega 3 at the ratio of 1:3, which is suitable for the human body.

So, this research interest has been paid to compare the hemp seed oil from 2 different extraction methods of conventional and soxhlet extraction using 3 other extraction solvents of water, n-hexane, and petroleum ether to know the most effective extraction method and appropriate extraction solvent for recommending further research and product development.

MATERIALS AND METHODS

Material

Hemp seed (*Cannabis sativa* L.) of RPF 1 variety (Figure 1) was cultivated by the Highland Research and Development Institute (Public Organization), Thailand, in 2020, and seeds were harvested from November to December 2020. The moisture content of the hemp seeds was determined according to be 10%, and seed purity was recorded at 98% (Pinmanee, 2019). After being harvested, hemp seeds were kept at room temperature until their use for the experiments.

Figure 1. Hemp seed (*Cannabis sativa* L.) RPF 1

Composition analysis

Crude protein content of Hemp seed was determined by the Kjeldahl method using protein conversion factor (N x 6.25). Moisture and ash contents were determined using AOAC official methods (Feldsine et al., 2002). Fat content was measured by using Fat Analyzer: Soxhlet Extraction Method (FOSS, ST243 Soxtec). Crude Fiber content was determined using AOAC official methods (Feldsine et al., 2002). Total carbohydrates, including crude fiber, were calculated by then.

Conventional extraction method

Initial oil content in hemp seed was measured by a separatory funnel with water, n-hexane, and petroleum ether; 1 g of ground hemp seeds was extracted with 50 ml solvent until totally depleted (Lavenburg et al., 2021). The whole process took 5 min, and each extraction experiment was conducted three times. The resulting suspension was filtered and removed solvent in a water bath at 80°C and measure the color of the solution. The oil obtained was weighed, and the yield was calculated. Determination was done in triplicate. The extraction yield and efficiency were calculated by the following Eq's:

$$\text{Oil extraction yield (\%)} = \frac{\text{Mass of extracted oil (g)}}{\text{Mass of sample (g)}} \times 100$$

Soxhlet extraction method

Initial oil content in hemp seed was measured by automatic soxhlet extraction systems with water, n-hexane, and petroleum ether; 1 g of ground hemp seeds was extracted with 50 ml solvent until totally depleted (Porto et al., 2013). The whole process took 30 min at 105 °C. Each extraction experiment was conducted three times. The resulting suspension was filtered and removed solvent in a water bath at 80 °C, then the color of the solution was measured. The oil obtained was weighed, and the yield was calculated. Determination was done in triplicate. The extraction yield and efficiency were calculated by the following Eq's

$$\text{Oil extraction yield (\%)} = \frac{\text{Mass of extracted oil (g)}}{\text{Mass of sample (g)}} \times 100$$

Color analysis

Color measurements of extracted oil_were performed using a Hunter Lab colorimeter (Color Flex EZ, ASTM E380). The instrument was pre-calibrated with a standard white and blackboard before sample measurement. The color parameters were expressed in terms of L* (lightness), a* (red/green), and b* (yellow/blue) values.

Statistical analysis

The experiments were repeated three times. The experimental results obtained were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range test procedures for SPSS. The significance of differences was defined at a 95% confidence level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Proximate composition analysis

The result of proximate analysis of hemp seed (*Cannabis sativa* L.) RPF 1 is shown in Table 1. There is a composition of 5.81% moisture content, 5.12% ash, 36.87% crude protein, 24.43% crude fat, 12.04% carbohydrate, and 0.65 water activity. According to the experimental results, especially protein content contained in analyzed hemp seeds shows higher content compared to those in egg protein (12.5%) (Rhault-Godbert *et al*., 2019), in Brazil nut seed (14.47%) (Lima, 2021), in almond seed (24.95%) (Lima, 2021), and in soy seed (14.8%) (Voleka *et al*., 2018).

Table 1. Proximate analysis of hemp seed (*Cannabis sativa* L.) RPF 1.

Parameter	Contain in Hemp seed
Moisture (%)	5.81±0.25
Ash (%)	5.12±0.08
Protein (%)	36.87±0.75
Fat (%)	24.43±0.15
Fiber (%)	12.04±2.06
Carbohydrate (%)	27.75±0.54
Water activity, Aw	0.65±0.00

Conventional and Soxhlet extraction analysis

The extraction yield and efficiency of different extraction methods and solvents were calculated (Table 2). The results showed that conventional extraction of hemp seed oil extracted by n-hexane shows the highest amount of hemp seed oil with a percent yield of 18.78%, followed by petroleum ether of 17.06%, and water of 4.56%, respectively. Additionally, the soxhlet extraction of hemp seed oil extraction by petroleum ether shows the highest amount of hemp seed oil with a percent yield of 24.44%, followed by n-hexane of 21.65% and water of 1.27%, respectively. As the results of hemp seed oil contents from different extraction solvents showed a significant difference at the 95% confidence level (P≤0.05) due to the polarity difference of the extraction solvent. According to the experimental results, it showed that oil content in RPF 1 hemp seed is higher content compared to the oil content in other seeds such as soy seed (19%) or sunflower seed (24%) (Voleka et al., 2018). Also, soxhlet extraction is one of the effective methods used for oil extraction, as shown in some previous studies. For example, Hu et al. (2021) reported that soxhlet extraction has oil yield higher than microwave-assisted extraction of *Sapindus mukorossi* seed and Eikani et al. (2012) reported that soxhlet extraction has oil yield higher than cold pressing in pomegranate seed. The colors of hemp seed oil extracted by different extraction methods and solvents are shown in Table 3. There was a significant difference (P≤0.05) in L*, a*, and b*, due to the pigment which was extracted by each solvents' ability.

Table 2. Hemp seed oil obtained from different extraction methods and solvents

Solvents	Conventional extraction method (% Yield)	Soxhlet extraction method (% Yield)
Water	4.56 ±0.07 ^{cA}	1.27±0.01 ^{cB}
n-hexane	17.06±0.11^{bB}	21.65±0.01^{bA}
petroleum ether	18.78±0.02^{aB}	24.44±0.01^{aA}

Remark: a-c shows significant difference at 95% confident level (P≤0.05) in column A-B shows significant difference at 95% confident level (P≤0.05) in row

Table 3. Colors of hemp seed oil obtained from different extraction methods and solvents

Solvents	Conventional extraction method			Soxhlet extraction method		
	L*	a*	b*	L*	a*	b*
Water	5.53^{c}	-0.41^{a}	0.07^{c}	38.22^{a}	-2.06^{c}	-5.12^{c}
n-hexane	7.94^{b}	-0.71^{b}	0.66^{a}	12.06^{b}	-1.13^{b}	2.42^{b}
petroleum ether	8.42^{a}	-0.76^{c}	0.33^{b}	3.51^{c}	-0.32^{a}	0.75^{a}

Remark: a-c shows significant difference at 95% confident level (P≤0.05) in column

CONCLUSIONS

The differences in extraction methods and solvents show differences in extraction efficiency in hemp seed oil extraction. According to the experimental results, soxhlet extraction method trends to be a more effective method than the conventional method, and petroleum ether works better as an extraction solvent in both conventional and soxhlet methods compared to those of water and n-hexane. So, it was concluded that the suitable and recommended method for getting a higher yield of hemp seed oil from RPF 1 hemp seed is the soxhlet extraction method using petroleum ether as a solvent.

ACKNOWLEDGMENTS

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REFERENCES

Deferne, J.L., and Pate, D. W. 1996. Hemp seed oil: A source of valuable essential fatty acids. Journal of the International Hemp Association. 3: 4-7.

Eikani, M.H., Golmohammada, F., and Homami, S.S. 2012.Extraction of pomegranate (*Punica granatum* L.) seed oil using superheated hexane. Food and Bioproducts Processing. 90: 32-36.

Feldsine, P., Abeyta, C., and Andrews, W.H. 2002. Methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. Journal of AOAC international. 85: 1187-1200, https://doi.org/10.1093/jaoac/85.5.1187.

Hu, B. Xi, X. Li, H. Qin, Y. Li, C. Zhang, Z. Liu, S., and Luo, O. 2021 A comparison of extraction yield, quality and thermal properties from *Sapindus mukorossi* seed oil between microwave assisted extraction and Soxhlet extraction. Industrial Crops & Products 161: 113185.

Lavenburg, V.M., Rosentrater, K.A., and Jung, S. 2021. Extraction methods of oils and phytochemicals from seeds and their environmental and economic impacts. Processes. 9: 1839. https://doi.org/10.3390/pr9101839.

Lima, D.S. 2021. Technological quality and sensory acceptability of nutritive bars produced with Brazil nut and Baru almond coproducts. LWT- Food Science and Technology. 137: 110467.

Pinmanee, S. 2019. Hemp varieties and legally certified seeds. The Highland Research and Development Institute (Public Organization). https://www.hrdi.or.th/articles/detail/47 (in Thai)

Porto, C.A., Porretto E., and Decorti, D. 2013. Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. Ultrasonics Sonochemistry. 20: 1076–1080.

Rehault-Godbert, S., Guyot, N., and Nys, Y. 2019. The golden egg: Nutritional value, bioactivities, and emerging benefits for human health. Nutrients. 11(3): 684. https://doi.org/10.3390/ nu11030684

Voleka , Z., Ebeidb, T.A., and Uhlirova, L. 2018. The impact of substituting soybean meal and sunflower meal with a mixture of white lupine seeds and rapeseed meal on rabbit doe milk yield and composition, and the growth performance and carcass traits of their litters. Animal Feed Science and Technology. 236: 187-195.

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Effects of micro-nano bubbles and electrical conductivity of nutrient solution on the growth and yield of green oak lettuce in a hydroponic production system

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ABSTRACT

The purpose of the study was to evaluate the effect of using water with micro-nano bubbles (MNBs) and different electrical conductivity (EC) on the growth and yield of green oak lettuce in hydroponic systems. The green oak lettuce plantation was studied from December 2020 to February 2021 at the Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Thailand. The experimental design was a 2x3 factorial in CRD. Factors were: 1) giving MNBs water at 0 or 5 minutes weekly and 2) giving of EC (Electrical conductivity) at 0.3, 0.6, and 1.2 mS cm^{-1} in a deep flow technique (DFT) hydroponics system. The results showed that MNB treated water for 5 minutes affected the fresh and dry weight of the leaves and the total weight of green oak lettuce, and the different levels of EC had significant effects on the growth and yield of green oak lettuce. The interaction between MNBs and EC affected plant height, canopy width, leaf greenness values (SPAD), fresh leaf yield, shoot, and the total weight of green oak lettuce had significant differences. Moreover, concentrations of N, P, K, Ca, and Mg in the leaf increased with increasing EC. Those MNBs treatment increased N, P, K, and Ca but did not affect Mg.

Keywords: electrical conductivity, micro-nano bubbles, hydroponic, green oak lettuce

INTRODUCTION

Green oak lettuce, known scientifically as *Lactuca sativa* var. crispa L., is a leafy vegetable that is very popular in Thailand due to its potential of growing well in hydroponic systems. Hydroponics is a new technique to grow plants in a nutrient solution that increases growth, yield, income, profit, and crop number per year. (Siringam et al., 2014). The average result of outdoor lettuce in hydroponic systems or the Nutrient Film Technique (NFT) with the principle of a constant flow of liquid nutrient solution produced about 1.85 times plant yield higher than that in soil culture (Wattanapreechanon and Sukprasert, 2012). Moreover, the benefits of hydroponic vegetable cultivation can also control plant growth, reduce agricultural chemicals, and prevent contamination of chemicals from the soil, resulting in better yield, sanitation, and quality than conventional methods (Quy et al., 2018). Several factors are involved in the growth of plants in hydroponic systems, including sunlight, water, temperature, plant nutrients, pH, and EC. Moreover, the concentrated nutrient solutions with regulated pH and EC also need to be kept at appropriate levels for each type of vegetable or plant. Too much or too little pH and EC will affect the quality, growth, and yield of the vegetable (Quy et al., 2018, Phaengkieo et al., 2019). Aside from nutrients in solution, oxygen content in water is one of the main factors affecting the growth of plants and vegetables. The MNBs are technology capable of making tiny air bubbles of 100 to 200-nanometer diameter. These tiny bubbles effectively increase the oxygen content in water, and with MNBs, the nutrient solution could also improve vegetative growth (Jiang et al., 2016, Sritontip et al., 2019a). Therefore, the objective of this experimental study was to evaluate the effect of MNBs water and EC on the physiological character, yield, and leaf nutrient concentration of green oak lettuce produced in a hydroponic system.

MATERIALS AND METHODS

The green oak lettuce was planted from December 2020 to February 2021 at the Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Thailand. The experiment was assigned in a factorial arrangement in a completely randomized design (CRD) with two factors, including applied MNBs for water at 0 and 5 minutes weekly for five consecutive weeks. It used EC with three levels: 0.3, 0.6, and 1.2 mS cm^{-1}, respectively. There were four replications each. The nutrient solution was modified from Hoagland and Arnon's (1952) and Huett (1993) formulas. The stock solution in 1 liter of water consisted of $Ca(No_3)_2$ $4H_2O$, KNO_3, $NH_4H_2PO_4$, KH_2PO_4, $MgSO_4$•$7H_2O$, Fe-EDTA, $MnSO_4$ H_2O, $ZnSO_4$•$7H_2O$, $CuSO_4$ $5H_2O$, H_3BO_3' and $(NH_4)_6Mo_7O_{24}$•$7H_2O$. All crops were grown in the non-circulating hydroponic growing method (Deep flow technique, DFT) (Kratky, 2009). Green oak lettuce was planted in boxes 25x29x10 centimeters in size that had the capacity of 3 liters of nutrient solution per box. The polystyrenes were kept floating on the nutrient solution to support the plants. The pH of the nutrient solution was maintained within the range of 6.5 by adding sulfuric acid. Oxygen was provided through an air pump generator. Every week air was supplied to the MNBs water for 0 and 5 minutes and the nutrient solution was added to the box after bubbling the air. The air micro-nano bubbles used model KVM-25 which were water flow rate of 25 L/min, airflow rate of 2 L/min, operation pressure 0.25-0.4 MPa, and 0.75 kW motor power; having air nanobubbles in the range of 107-108 bubbles/mL measured by Horiba-960A laser bubble analyzer that developed by the unit of excellence in the High Voltage Plasma and Micro/Nano Bubble Application to Agriculture and Aquaculture, Rajamangala University of Technology Lanna (RMUTL), Chiang Mai, Thailand. The dissolved oxygen (DO) of the nutrient solution was measured by waterproof, model DO600, Eutech Instrument, Singapore; EC was controlled with EC meter, model EC 59, Martini Instrument, Romania. The physiological character attributes of green oak lettuce in a hydroponic system were measured, including plant height, canopy width, leaf number per plant, root length, leaf greenness value (SPAD), fresh leaf, dry leaf, shoot, root, and total weight. Growth parameters of green oak lettuce were recorded weekly for five weeks from the plant-grown period until harvesting. The fresh weight of leaves, shoot, roots, and the total weight were recorded after 45 days of growth. The material was kept in an oven at 70 ºC in a paper bag for dry weight. Once dry, it was then taken out of the oven and recorded. The leaf nutrient concentration was analyzed. The samples were washed and dried at 70 ˚C for 48 hours and milled. Leaf nitrogen was determined using a micro-Kjeldahl digestion solution. The digested solution was diluted before colorimetric analysis using the indophenol reaction (Novozamsky et al., 1974). Phosphorus was determined by dry digestion followed by the vanadomolybdate method (Walinga et al., 1995), and K, Ca, and Mg by dry digestion and atomic absorption spectroscopy (AA-6401F, Atomic absorption flame emission spectrophotometer, Shimadzu, Tokyo, Japan) (Kalra, 1998; Walinga et al., 1995). The statistical data were analyzed for Analysis of Variance. Statistical differences with P-values less than 0.05 and 0.01 were considered significant, and highly significant, respectively; the means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Different EC levels in the nutrient solution at 0.6 and 1.2 $mS.cm^{-1}$ influenced plant height and canopy width of green oak lettuce at weeks 3, 4, and 5 (Figures 1 and 3). The 5-minutes of MNBs significantly affected plant height at week 5 (Figure 2) and canopy width at weeks 2 and 3 (Figure 4). There were interactions between the solution concentrations and MNBs water on height growth at weeks 4 and 5 (Figure 5) and canopy width at weeks 2, 3, 4, and 5 (Figure 6).

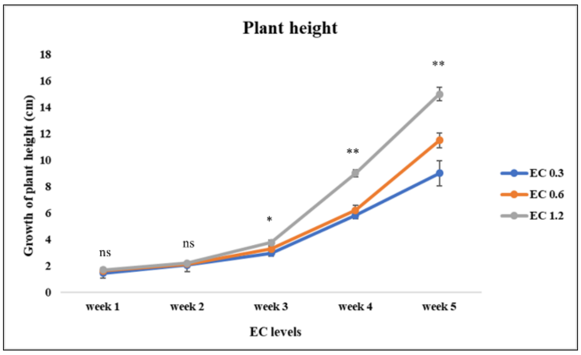

Figure 1. Effect of EC levels on plant height.

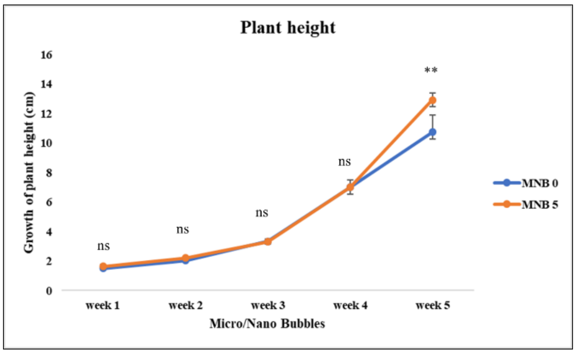

Figure 2. Effect of MNBs on plant height.

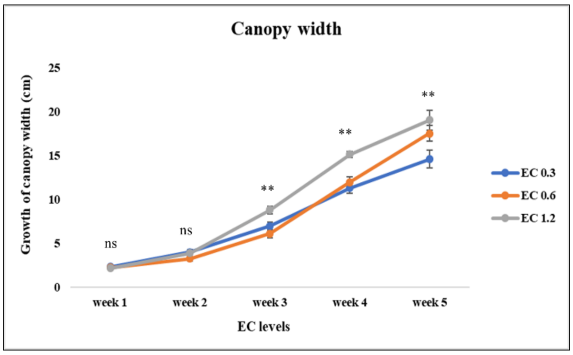

Figure 3. Effect of EC levels on canopy width.

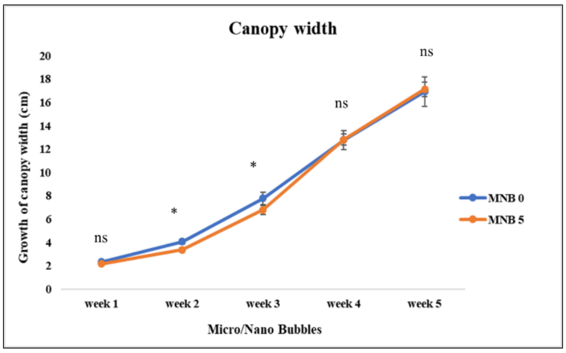

Figure 4. Effect of MNBs on canopy width.

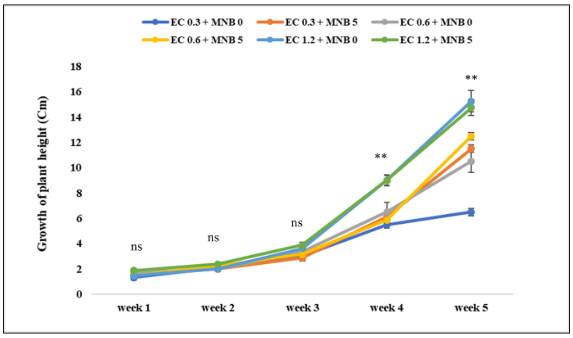

Figure 5. Combination between EC levels and MNBs on plant height.

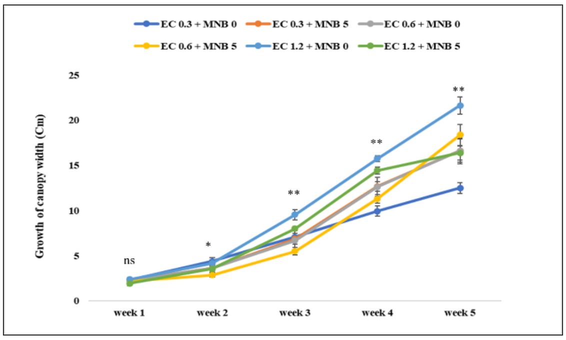

Figure 6. Combination between EC levels and MNBs on canopy width.

At 1.2 mS/cm^{-1}, the EC concentration level affected the leaf number at weeks 2, 4, and 5 (Figure 7). When using 0 and 5 minutes of MNBs water, the number of leaves did not change significantly (Figure 8) but interacted with each other. At the EC concentration of 1.2 mS.cm$^{-1,}$ the most extended root length was found at weeks 3 and 4 (Figure 9). The root length when 5 minutes of MNB water were used was significant at week 4 (Figure 10). The interaction of the solution concentrations and MNBs water affected leaf number at weeks 2 and 5 (Figure 11) and root length at weeks 3 and 4 (Figure 12).

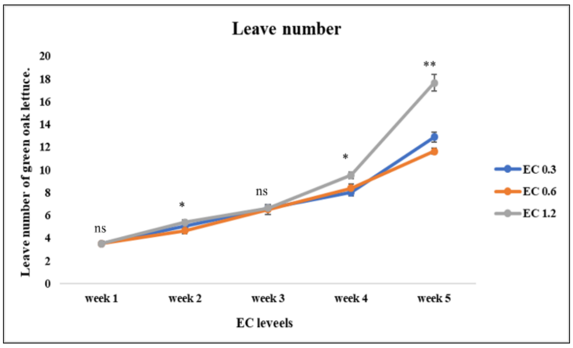

Figure 7. Effect of EC levels on leaf number.

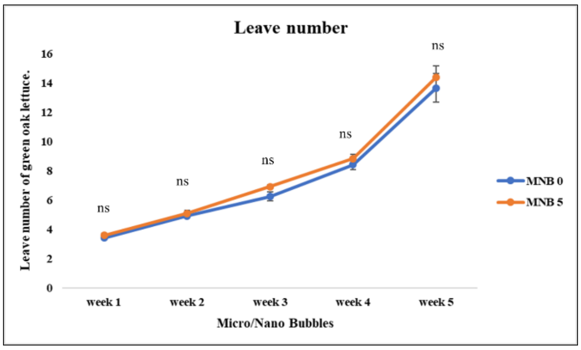

Figure 8. Effect of MNBs on leaf number.

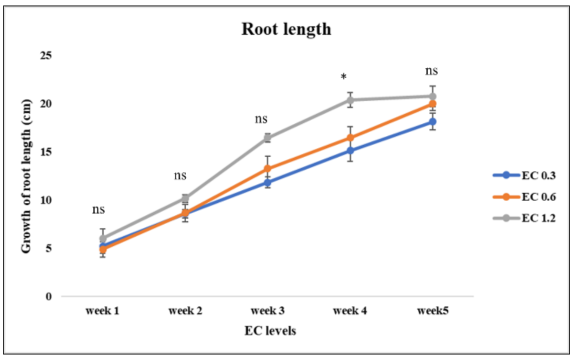

Figure 9. Effect of EC levels on root length

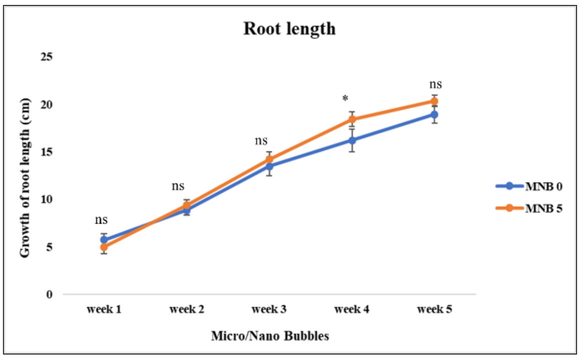

Figure 10. Effect of MNBs on root length.

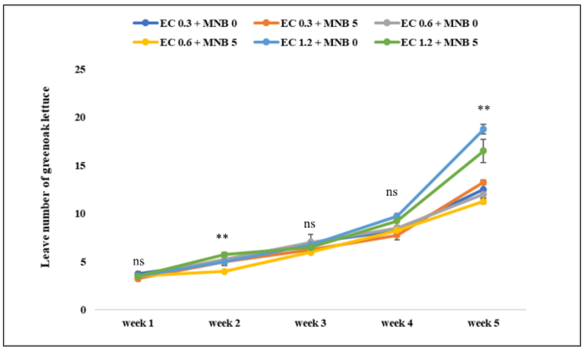

Figure 11. Combination between EC levels and MNBs on leaf number.

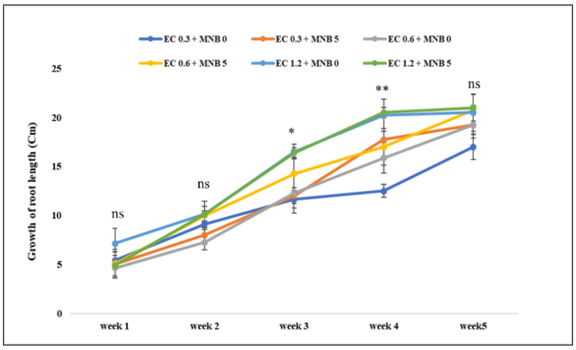

Figure 12. Combination between EC levels and MNBs on root length.

The SPAD unit of green oak lettuce was affected at EC 0.6 and EC 1.2 mS cm^{-1} at weeks 2, 3, and 4 (Figure 13), and significantly after using 5 minutes of MNBs water at week 4 (Figure 14). The solution concentrations and MNBs water interacted at weeks 3 and 4 (Figure 15).

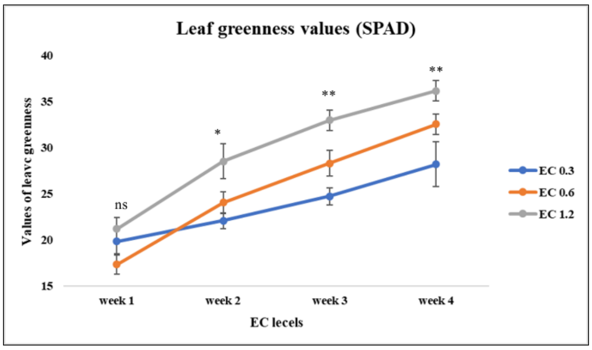

Figure 13. Effect of EC levels on SPAD.

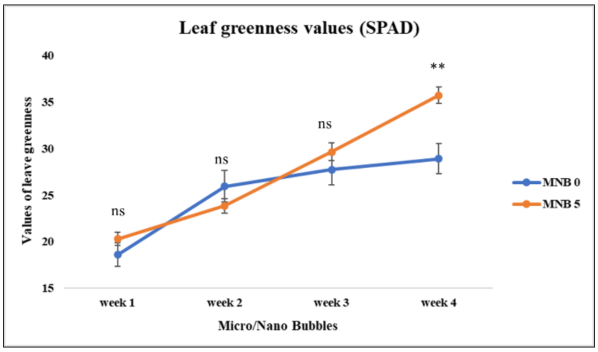

Figure 14. Effect of MNBs on SPAD.

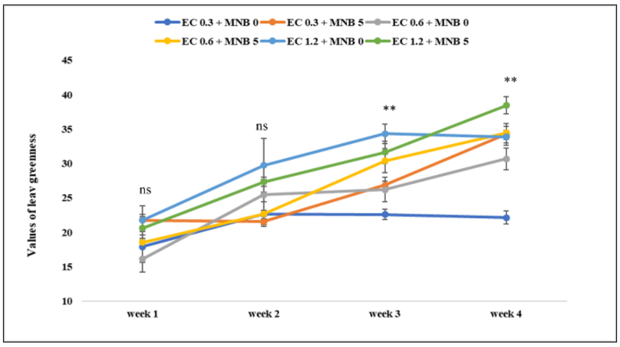

Figure 15. Combination between EC levels and MNBs on SPAD.

At an EC concentration of 1.2 mS cm^{-1}, the yield of the fresh and dry weight of leaf, shoot, root, and the total weight of green leaf oak lettuce was higher than for EC 0.3 and 0.6 mS cm^{-1}, see Figures 16 and 19. Using water with MNBs for 5 minutes produced a higher fresh and dry weight of the leaves and a higher total weight of green oak lettuce (Figures 17 and 20). The interaction of EC and MNB water gave significant differences between the fresh weight of leaves, shoots, root and the total weight (Figure 18). However, this interaction was not significant for the dry weight of the root (Figure 21).

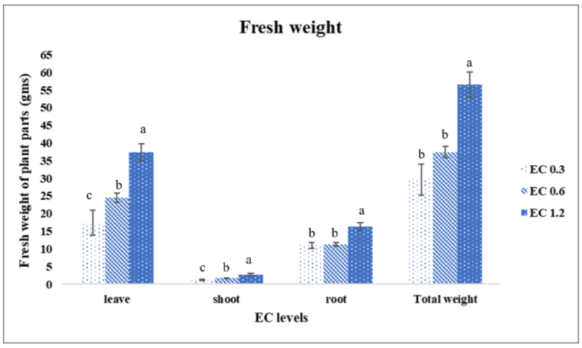

Figure 16. Effect of EC levels on fresh weight.

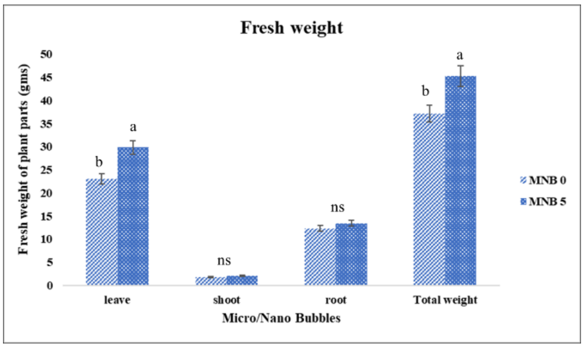

Figure 17. Effect of MNBs on fresh weight.

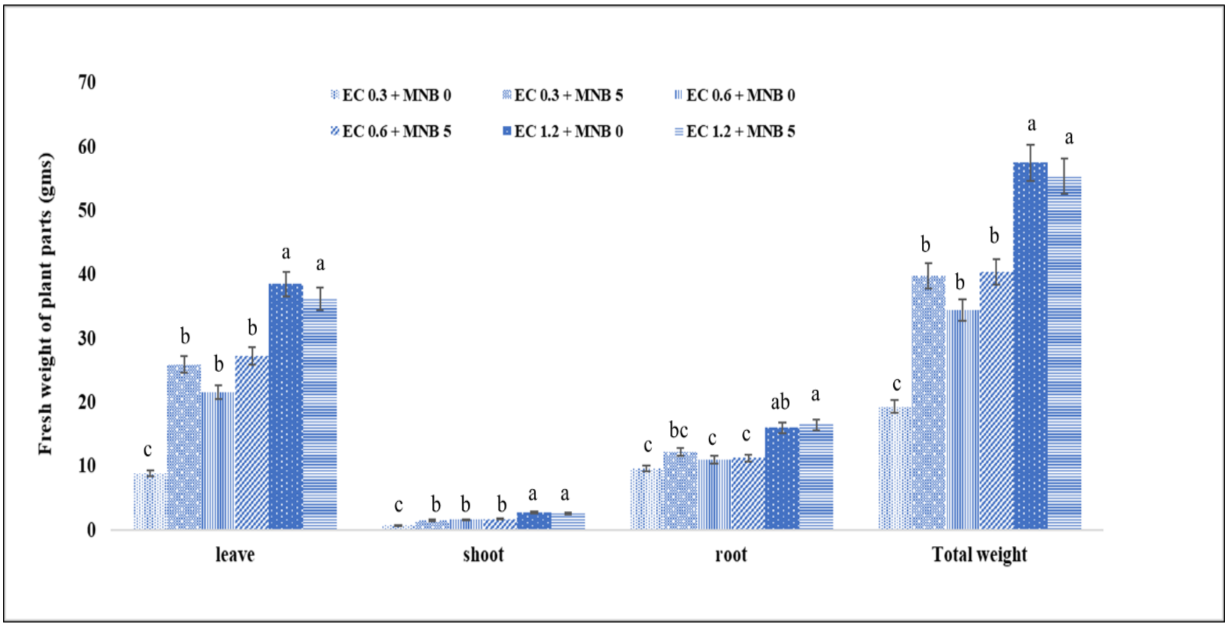

Figure 18. Combination between EC levels and MNBs on fresh weight.

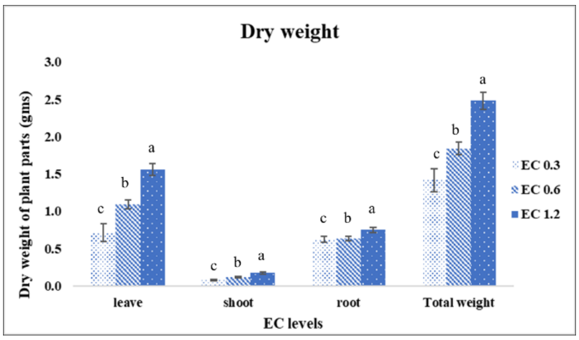

Figure 19. Effect of EC levels on dry weight.

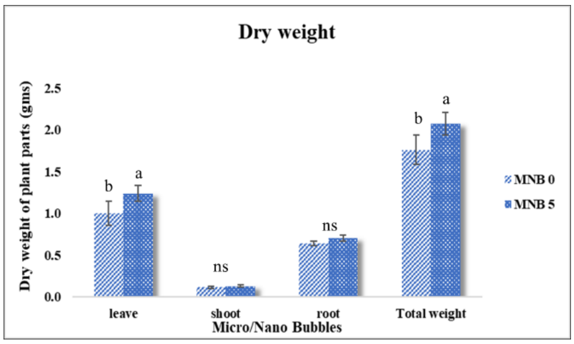

Figure 20. Effect of MNBs on dry weight.

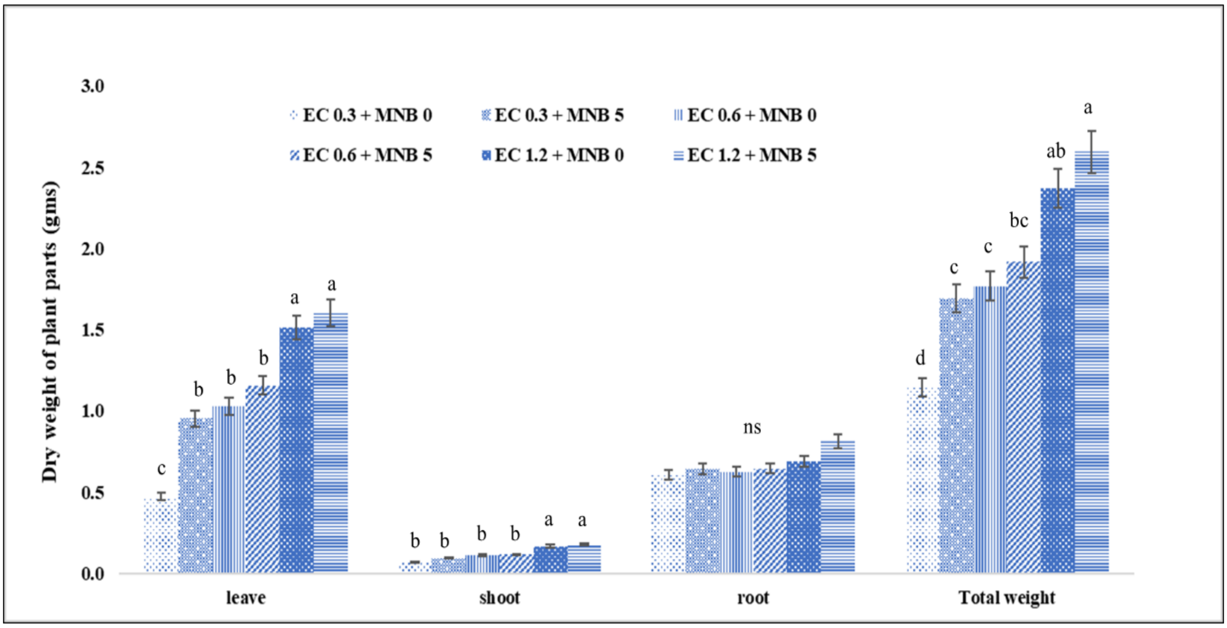

Figure 21. Combination between EC levels and MNBs on dry weight.

At electrical conductivities of 0.3 to 1.2 mS cm^{-1} the dissolved oxygen in the nutrient solution of the culture showed similar patterns (Figure 22). However, a 5-minute MNBs treatment increased the dissolved oxygen to levels not reached with MNBs-untreated water (Figure 23).

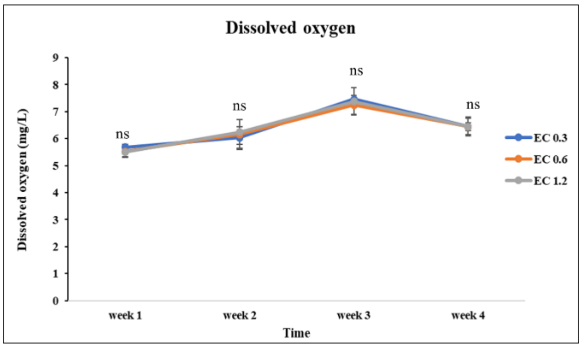

Figure 22. Effect of EC levels on dissolved oxygen

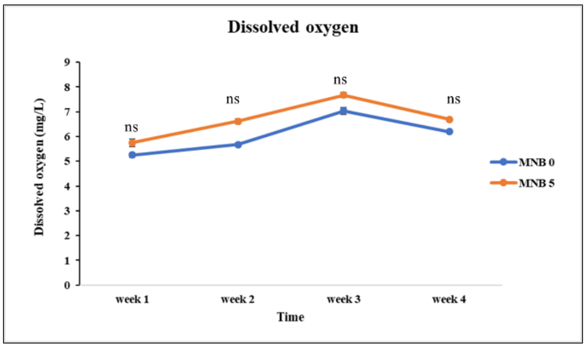

Figure 23. Effect of MNBs on dissolved oxygen.

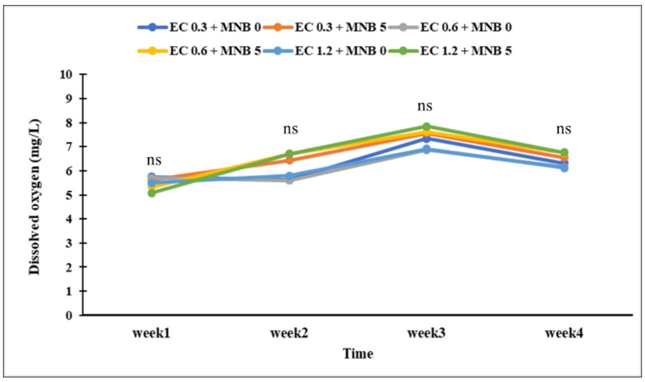

Figure 24. Combination between EC levels and MNBs on dissolved oxygen.

The concentrations of nitrogen, phosphorus, potassium, calcium, and magnesium in green oak lettuce plant tissue increased when the EC was increased from 0.3-1.2 mS cm^{-1}. Furthermore, a MNBs treatment increased the leaf nutrient concentration of nitrogen, phosphorus, potassium, and calcium to higher than a non-MNBs treatment, while magnesium and phosphorous uptake levels remained similar. There was an interaction between EC and MNBs on the nitrogen concentration. At 0.3-0.6 mS cm^{-1} were more effective of MNBs treatment that could induce leaf nitrogen concentration (Table 1).

Table 1. Effect of EC levels and period of MNBs on leaf nutrient concentration after treatments.

Factors	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)
EC (A)					
0.3 mS cm^{-1}	3.57^{c}	0.08^{c}	1.92^{c}	5.72^{b}	0.11^{b}
0.6 mS cm^{-1}	4.44^{b}	0.12^{b}	2.95^{b}	6.14^{ab}	0.14^{ab}
1.2 mS cm^{-1}	4.99^{a}	0.14^{a}	3.56^{a}	7.69^{a}	0.15^{a}
MNBs (B)					
0 minute	4.10^{b}	0.10^{b}	2.41^{b}	6.13^{b}	0.13
5 minutes	4.56^{a}	0.12^{a}	3.20^{a}	6.91^{a}	0.14
A*B					
0.3 mS cm^{-1}	3.05^{d}	0.06	1.37	5.57	0.10
0.3 mS cm^{-1} + MNBs	4.08^{c}	0.09	2.46	5.88	0.12
0.6 mS cm^{-1}	4.26^{bc}	0.11	2.78	5.65	0.14
0.6 mS cm^{-1} + MNB	4.62^{ab}	0.12	2.89	6.46	0.13
1.2 mS cm^{-1}	4.98^{a}	0.12	3.36	7.33	0.14
1.2 mS cm^{-1} + MNB	4.99^{a}	0.15	4.02	8.22	0.16
A	*	*	*	*	*
B	*	*	*	*	ns
A x B	*	ns	ns	ns	ns

*Means within the column followed by the different letters were significantly different (P< 0.05), ns =not significant.

The effect of water with MNBs and EC levels on the growth and yield of green oak lettuce in hydroponic systems was studied. EC levels of 0.5-2.0 mS cm^{-1} enhanced growth and yield in green oak lettuce (Department of Agriculture Extension, 2015). Maximum growth was achieved with solution concentrations between 1.2 and 4.8 mS.cm^{-1} (Albornoz and Lieth, 2015). The Air MNBs water induced plant growth in green oak lettuce because MNBs water increased DO in the medium and enhanced the oxidation effect of surface water (Oshita and Liu. 2013, Takahashi, 2005; Liu and Tang, 2019). Jiang et al. (2016) reported that the introduction of oxygenated MNB into the water significantly improved the DO content, resulting in increased plant growth, product yield, and quality of the lettuce. The lettuce grown in the nano-bubble system had a healthier appearance, plant height, canopy width and longer roots than the same plant grown in a conventional hydroponic system (Phaengkieo et al., 2019). Furthermore, the EC levels of an experiment in 'Green Butter' found that the yield was 75 g less at 1.4 mS.cm^{-1} when compared with 1.8 mS.cm^{-1} (Samarakoon et al., 2020). This experiment indicated that as the EC levels increased from 0.3-1.2 mS cm^{-1}, the N, P, K, and Ca in leaf tissue were greater in increasing EC. The lettuce plant growth and yield were higher than lower EC. The nutrient solution was total ionic concentration from 16 essential elements (Trejo-Téllez and Gómez-Merino, 2021). Furthermore, in the research on dill tissue (*Anethum graveolens* 'Fernleaf'), the concentration of N, K, S (sulfur), Fe (iron), and B (boron) climbed with increasing EC. At the same time, calcium and magnesium were negatively affected (Currey et al., 2019). Moreover, EC of the nutrient solution in all three basil species showed that the SPAD increased with increasing EC, and in holy basil tissue, N and P increased when the EC rose from 0.5 to 3.0 mS cm^{-1} (Walters and Currey, 2018)

Using water treated with MNBs resulted in a positive effect when the MNBs were tiny bubbles smaller than 50 micrometers and 200 nanometers. Air MNBs can stay for long periods. (Agarwal et al., 2011; Takahashi, 2015). The lettuce can be sensitive to MNBs treatments and improve plant growth because of the small size of gas bubbles, long residence time in the water, the ability to generate hydroxyl radicles, and tremendous negative electronic changes (Agarwal et al., 2011; Zhang et al., 2020; Tsuge, 2015). Takahashi (2015) reported that the microbubbles were electrical double layers with a negative charge in above pH conditions and positive charge in strong acid conditions. The growth and development were stimulated with MNBs. That was possibly why the positive ions in the nutrient solution were transported preferentially up the root system and DO enrichment in a nutrient solution. The microbubbles in the nutrient solution could induce plant growth and enhance the development of lettuce under hydroponic conditions (Park and Kurata, 2009; Park et al., 2010). Furthermore, fine bubbles or MNBs enhanced seed germination and root length of Chinese celery and sweet corn. MNBs promoted vegetative growth in melons but did not affect fruit quality (Sritontip et al., 2019b). Moreover, ultrafine bubbles enhanced the plant growth of soybean seedlings (Iijima et al., 2020). MNBs characterize by small specific surface areas and long residence times in water (Lui and Tang, 2019; Lui et al., 2019). This research indicated 5 minutes MNBs could stimulate the growth and development of green oak lettuce. Besides, EC levels of 1.2 mS cm^{-1} appropriated nutrient solution concentration of green oak lettuce.

CONCLUSIONS

This experiment studied the effect of oxygen micro-nano bubble (MNBs) water generated through an air pump. Supplying MNB water for 5 minutes to green oak lettuce grown in a hydroponic system increased the fresh and dry weight of the leaves and the total plant weight. Three concentrations of EC, 0.3, 0.6, and 1.2 mS cm^{-1}, gave significant differences and affected the growth and yield of green oak lettuce. However, the EC concentration of 1.2 mS cm^{-1} was the greatest of all treatments. An interaction between the MNBs and EC was also observed, resulting in improved plant height, canopy width, leaf greenness values (SPAD), the yield of fresh leaves, shoot, and the total weight of green oak lettuce. Increasing the EC also increased N, P, K, Ca, and Mg in the leaves.

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REFERENCES

Agarwal, A., Ng, W. J., and Liu., Y. 2011. Principle and applications of microbubble and nanobubble technology for water treatment. Chemosphere 84(9): 1175-1180.

Albornoz, F., and Lieth., J. H. 2015. Over fertilization limit lettuce productivity because of osmotic stress. Chilean Journal of Agricultural Research. 75(3): 284-290.

Currey, C.J., Walters, K.J., and Flax, N.J. 2019. Nutrient solution strength does not interact with the daily light integral to affect hydroponic cilantro, dill, and parsley growth and tissue mineral nutrient concentrations. Agronomy 9(7): 389.

Department of Agriculture Extension. 2015. Hydroponic plantation. Ministry of Agriculture and Cooperatives, Thailand.

Hoagland, D. R., and Arnon, D.I. 1952. The water-culture method for growing plants without soil. California Agricultural Experimental Station. The 2nd editions. Circ. 347. California.

Huett, D.O. 1993. Managing nutrient solutions in hydroponics. NSW Agriculture and Horticulture Research & Development Corporation, Wollongbar, New South Wales.

Iijima, M., Yamashita, K., Hirooka, Y., Ueda, Y., Yamane, K., and Kamimura, C. 2020. Ultrafine bubbles effectively enhance soybean seedling growth under nutrient deficit stress. Plant Production Science. 23 (3): 366-373. https://doi.org/10.1080/1343943X.2020.1725391.

Jiang C., Zhao, S., Song, W., Yamaguchi, T., and Riskowski., G.L. 2016. Effect of micro/nano bubble water on growth, yield and quality of lettuce under substrate cultivation. International Agricultural Engineering Journal. 25(3): 1-8.

Kalra, Y.P. 1998. Handbook of reference methods for plant analysis. CRC Press, Boca Raton.

Kratky, B.A. 2009. Three non-circulating hydroponic methods for growing lettuce. Acta.Hort. 843: 65-72.

Liu, C., and Tang. Y., 2019. Application research of micro and nano bubbles in water pollution control. E3S Web Conference 136: 1-3.

Liu, Y.X., Zhou, Y.P., Wang, T.Z., Pan, J.C., Zhou, B., Muhammad, T., Zhou, C.F., and Li, Y.K. 2019. Micro-nano bubble water oxygation: Synergistically improving irrigation water use efficiency, crop yield and quality. J. Clean. Prod. 222: 835–843. https://doi.org/10.1016/j.jclepro.2019.02.208.

Park, J.S., and Kurata., K. 2009. Application of microbubbles to hydroponics solution promotes lettuce growth. Hort. Technology. 19(1): 212-215.

Park, J.S., Ohashi, K., Kurata, K., and Lee, J.W. 2010. Promotion of lettuce growth by application of microbubbles in nutrient solution using different rates of electrical conductivity and under periodic intermittent generation in a deep flow technique culture system. Europ. J. Hort. Sci. 75(5): 198-203.

Phaengkieo, D., Chaoumead., A., Wangngon, B., and Chumnumwat., S. 2019. The application of nano bubble technology for DRFT hydroponics. Journal of Innovative Technology Research. 3(2): 33-41.

Novozamsky, I., Van Eck, R., Van Schouwenburg, J.C., and Walinga, I. 1974. Total nitrogen determination in plant material by means of the indophenol blue method. Netherlands Journal of Agricultural Science 22: 3-5.

Oshita, S., and Liu, S., 2013. Nanobubble characteristics and its application to agriculture and foods. In: Proceedings of AFHW 2013, International Symposium on Agri-Foods for Health and Wealth. August 5-8, 2013, Golden Tulip Sovereign Hotel, Bangkok, Thailand. p.23-32.

Quy, N. V., Sinsiri, W., Chitchamnong, S., Boontiang, K., and Kaewduangta. W. 2018. Effects of electrical conductivity (EC) of the nutrient solution on growth, yield and quality of lettuce under vertical hydroponic systems. Khon Kaen Agricultural Journal. 46(3): 613-622.

Samarakoon, U., Palmer, J., Ling, P., and Avenue, M. 2020. Effects of electrical conductivity, pH, and foliar application of calcium chloride on yield and tipburn of *Lactuca sativa* grown using the nutrient-film technique. Hortscience 55(8): 1265–1271.

Siringam, K., Theerawipa, K., and Hlaihakhot., N. 2014. Effect of nutrient solution on growth of lettuce (*Lactuca satica* L.) cultivated under hydroponic system. Journal of Science and Technology. 22(6): 828-836.

Sritontip, C., Dechthummarong, C., Thonglek, V., Khaosumain, Y., and Sritontip, P. 2019a. Stimulation of seed germination and physiological development in plants by high voltage plasma and fine bubbles. International Journal of Plasma Environmental Science & Technology 12(2): 74-78.

Sritontip C., Sritontip, P., Changjeraja., S., Khaosumain, Y., Amthong., S., Thonglek, V., and Tana, R. 2019b. Effects of bubbles on growth and yield of red oak lettuce grown in nutrient solution in summer season. In: The 10th RMUTs International Conference "Creative Innovation and Technology for Sustainable Agriculture. p. 27-31.

Takahashi, M. 2005. Potential of microbubbles in aqueous solutions: electrical properties of the gas-water interface. J. Phys. Chem. B109: 21858-21864.

Takahashi, M. 2015. Nanobubbles: An introduction. In: H. Tsuge (ed). Micro-and nanobubbles fundamental and applications. CRC press, Taylor & Francis Group. Florida. p. 307-315.

Trejo-Téllez, L. I. and Gómez-Merino, F. C. 2012. Chapter 1: nutrient solutions for hydroponic systems. In: T. Asao (ed). Hydroponics – A standard methodology for plant biological researches. InTech. Croatia. p. 1-22.

Tsuge, H. 2015. Chapter 1: Characteristic of microbubbles. In: H. Tsuge (ed). Micro-and nanobubbles fundamental and applications. CRC press, Taylor & Francis Group. Florida. p.3-10.

Walinga, I., van der Lee, J.J., Houba, V.J.G., van Vark, W., and Novozamsky, I. 1995. Plant analysis manual. Springer, Dordrecht. 275 p.

Walters, K.J., and Currey, C.J. 2018. Effects of nutrient solution concentration and daily light integral on growth and nutrient concentration of several basil species in hydroponic production. HortScience 53: 1319-1325.

Wattanapreechanon, E. and Sukprasert, P. 2012. Development of soilless culture for crop production in Thailand. Kasetsart J. (Soc. Sci) 33: 475-485.

Zhang, M., L. Qiu and Guicai, L. 2020. Basic characteristics and application of micro-nano bubbles in water treatment. In: 4th International Workshop on Renewable Energy and Development (IWRED 2020). IOP Conf. Series: Earth and Environmental Science 510.

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Effects of leonardite and nutrient management on growth and yield of cowpea (*Vigna unguiculata* L. Walp.)

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ABSTRACT

A study on the effects of leonardite and nutrient management on growth and yield of cowpea (*Vigna unguiculata* L. Walp.) was established in the greenhouse from July to October 2021 at the Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang, Thailand. The experimental design was a completely randomized design (CRD) with nine treatments and ten replications: (1) control, (2) 500 kg/rai leonardite, (3) 1,000 kg/rai leonardite, (4) 500 kg/rai leonardite + 100% chemical fertilizer based on soil analysis, (5) 1,000 kg/rai leonardite + 100% chemical fertilizer based on soil analysis, (6) 500 kg/rai leonardite + 75% chemical fertilizer based on soil analysis, (7) 1,000 kg/rai leonardite + 75% chemical fertilizer based on soil analysis, (8) 100% chemical fertilizer based on soil analysis and (9) 75% chemical fertilizer based on soil analysis. The result showed that the combination of leonardite with chemical fertilizers based on soil analysis affected the vegetative growth and yield of cowpea. The application of 500 kg/rai of leonardite in combination with 100% chemical fertilizer based on soil analysis gave the highest plant height, canopy width, stem diameter, leaf width, leaf length, leaf greenness (SPAD), fresh weight, and dry weight of pod per plant. Therefore, applying leonardite with chemical fertilizers based on soil analysis could enhance cowpea growth and yield.

Keywords: leonardite, nutrient management, cowpea

INTRODUCTION

Cowpea, scientifically known as *Vigna unguiculata* L. Walp, is a type of legume that is easy to grow and can be planted all over the year. The different cowpea varieties are also a source of protein and other nutrients essential for fresh food pods or as dry seeds in the industry (Enyiukwu et al., 2018). Cowpea seeds contain many bioactive compounds: bioactive peptides, dietary fibers, polyphenols, anti-oxidants, some vitamins and minerals that have important nutritional value to human health (Khan et al., 2007; Goncalves et al., 2016; Jayathilake et al., 2018). In Thailand, cowpea is widely grown for fresh vegetable food in the northern and northeastern regions of the country (Benchasri et al., 2014). Department of Agricultural Extension (2019) reported that the situation of the planting year 2018/19, the most planting areas in 5 provinces of Thailand, which are Lampang, Nakhon Ratchasima, Chiang Mai, Surin, and Sakon Nakhon, with 526 rai of total planted area, 384,210 kilograms total yield, 861 kilograms yield per rai and 13.44 Baht selling price per kilogram.

Department of Land Development (2015) reported that the land was used for agriculture with improper management, such as using chemical fertilizers without adding any organic matter or organic fertilizer to the soil. Some areas of the cultivation of the plant were the repeated planting or growing of the same plant for many years. The problem was that the amount of organic matter and soil fertility could be reduced. In addition, most of the soil in Thailand has too low (<1.5 %) to medium (1.5-3.5 %) organic matter. Soil organic matter is essential for water holding, cation exchange capacity, the ability of nutrient release, and absorption of positive ions (Faculty of the Department of Soil Science, 2006; Pompranee, 2017). Leonardite is a natural oxidation product of lignite coal; it is created through the decomposition of plants and animals by chemical and biological processes (Totirakul et al., 2009). Leonardite has high contents of humic substances: fulvic acid, humic acid, and humin (Ratanaprommanee et al., 2016) and also has a large amount of organic matter, high cation exchange capacity, and contains many plant nutrients (Pochadom et al., 2013; Landrot et al., 2020). However, leonardite contains low plant nutrient contents that may not be enough to enhance plant growth and yield. Therefore, this study aimed to evaluate the effects of leonardite and leonardite in combination with chemical fertilizers on the growth and yields of cowpea.

MATERIALS AND METHODS

The effects of leonardite and nutrient management on growth and yield of cowpea were investigated. A cowpea plantation was established in the greenhouse from July to October 2021 at the Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Thailand. The experiment was assigned in a completely randomized design (CRD) with nine treatments and ten replications as follows:

(1) control

(2) 500 kg/rai leonardite

(3) 1,000 kg/rai leonardite

(4) 500 kg/rai leonardite + 100% chemical fertilizer based on soil analysis

(5) 1,000 kg/rai leonardite +100% chemical fertilizer based on soil analysis

(6) 500 kg/rai leonardite + 75% chemical fertilizer based on soil analysis

(7) 1,000 kg/rai leonardite + 75% chemical fertilizer based on soil analysis

(8) 100% chemical fertilizer based on soil analysis

(9) 75% chemical fertilizer based on soil analysis.

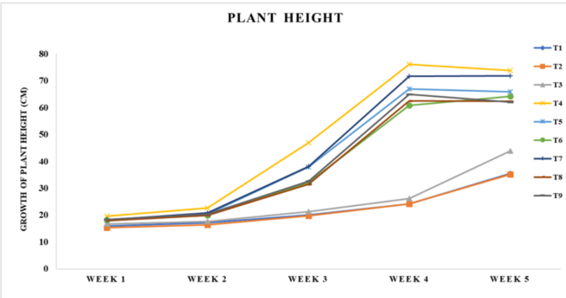
The soil sample of the experimental was collected at a depth of 0-20 centimeters from the Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang, Thailand, for analysis of some chemical properties. Soil chemical analysis contains the amount of organic matter (% OM) was 0.73, available phosphorus was 2.11 mg $kg^{-1,}$ and extractable potassium contents were 72.63 mg kg^{-1} with the soil pH (1:1) of 7.5. Leonardite was combined with the soil before growing the plants. Cowpea was planted in a pot with a size of 30 x 22.86 centimeters and had a capacity of 8 kilograms of soil per pot. Four seeds were planted per pot in the first week with a 1-2 centimeters depth. Seven days after planting, one healthy plant was left per pot. According to soil analysis results, chemical fertilizer formulas 46-0-0, 0-46-0, and 0-0-60 were mixed and used in the experiment. Fertilizer was applied by the conventional schedule two times during the growing period of cowpea, the first time at 7 days and the second time at 20 days after growing by sowing the soil around 10 centimeters from the stem and covering it with soil. Irrigation was performed with a drip irrigation system. Weed control was carried out by hand depending on weed density.

Measuring attributes of the physiology character of cowpea were recorded from 10 plants per treatment. Plant height, canopy width, and stem diameter were recorded every week after growing until harvesting. Leaf number per plant, leaf width, leaf length, leaf greenness values (SPAD), internode, and peduncle length were recorded during the flowering period. Fresh and dry weight of stems, leave, roots, and pod were harvested and recorded after planting for 56 days. The dry weight was recorded after being kept in the oven at 70 ºC for 72 hours.

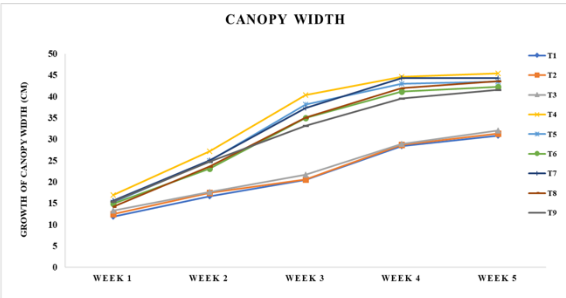
The data were analyzed for variances (ANOVA). Statistical differences with p-values less than 0.05 and 0.01 were considered significant, and the means were compared by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

The combination of 500 kg/rai leonardite with 100% chemical fertilizers based on soil analysis increased plant height, canopy width, and stem diameter at all planting periods after being treated with only leonardite (Figure 1)



a.



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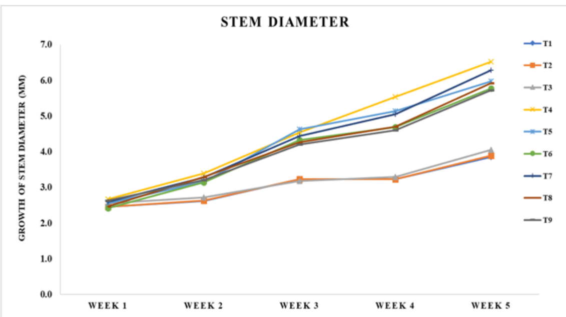

c.

Figure 1. Effect of leonardite and nutrient management on growth of cowpea (a) plant height, (b) canopy width, and (c) stem diameter

The number of leaves, leaf width and length, and leaf greenness (SPAD) were significantly different among the treatments (P≤0.01). The results showed that the highest number of leaves, leaf width and length, and leaf greenness were observed using 500 kg/rai leonardite combined with 100% chemical fertilizers based on soil analysis (Table 1).

During the flowering period, the internode and peduncle length was also significantly different among the treatments (P≤0.01). Internode and peduncle length of plants treated with only leonardite and untreated control was lower than an application with chemical fertilizers and leonardite with chemical fertilizers based on soil analysis (Table 1).

Table 1. Effect of leonardite and nutrient management on growth of cowpea during the flowering period

Treatments	Leaf number	Leaf width (cm)	Leaf length (cm)	SPAD	Internode (cm)	Peduncle length (cm)
Control	5.20^{b}	3.41^{c}	7.83^{d}	50.68^{c}	6.10^{b}	4.20^{b}
Leo 500	5.50^{b}	3.40^{c}	7.84^{d}	51.43^{c}	4.50^{b}	3.79^{b}
Leo 1,000	5.30^{b}	3.69^{bc}	7.90^{d}	52.33^{c}	6.47^{b}	4.70^{b}
Leo 500 + CF 100%	7.30^{a}	4.14^{a}	9.40^{a}	60.67^{a}	10.78^{a}	16.55^{a}
Leo 1,000 + CF 100%	7.00^{a}	3.89^{ab}	8.76^{cd}	58.26^{a}	11.75^{a}	15.22^{a}
Leo 500 + CF 75%	7.10^{a}	4.01^{ab}	8.87^{abc}	56.87^{ab}	10.32^{a}	15.69^{a}
Leo 1,000 + CF 75%	7.40^{a}	4.02^{ab}	9.29^{ab}	59.18^{a}	10.88^{a}	18.72^{a}
CF 100%	7.10^{a}	3.77^{b}	8.62^{c}	57.00^{ab}	12.00^{a}	18.01^{a}
CF 75%	7.00^{a}	3.87^{ab}	9.18^{abc}	53.46^{bc}	11.70^{a}	14.47^{a}
F-test	**	**	**	**	**	**
C.V. (%)	7.30	8.81	7.43	8.18	22.91	32.39

Leo- Leonardite, CF- Chemical Fertilizer, **, significant at P≤0.01 by DMRT

The combination of leonardite with chemical fertilizers based on soil analysis affected the fresh and dry weight of stems, leaves, and cowpea roots. The highest fresh and dry weight stem, leave, and root was obtained from the treatment of 500 kg/rai leonardite in combination with 100% chemical fertilizers based on soil analysis (Figures 2 and 3).

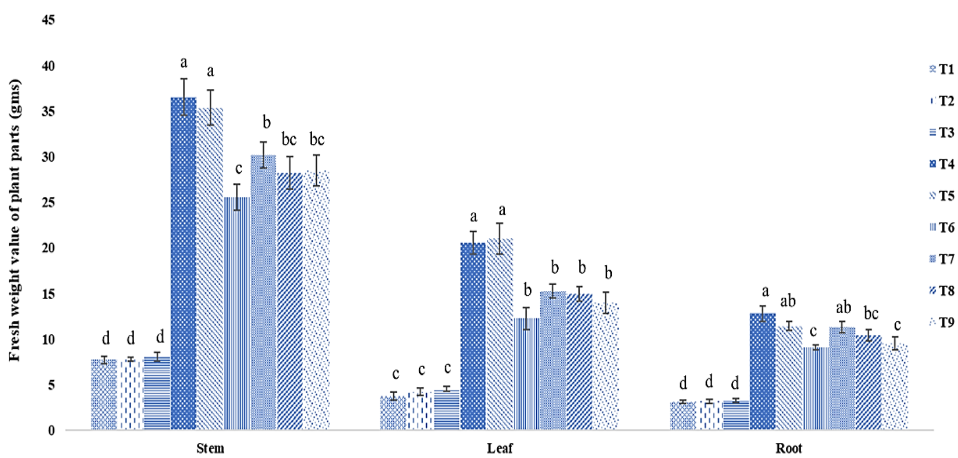

Figure 2. Effect of leonardite and nutrient management on fresh weight of stems, leave, and roots of cowpea

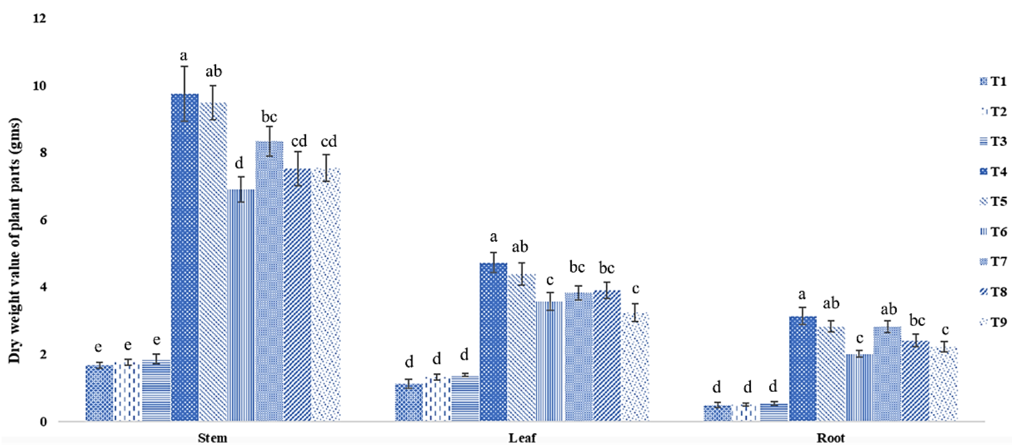

Figure 3. Effect of leonardite and nutrient management on dry weight of stems, leave, and roots of cowpea

The fresh and dry weights of pod per plant were significantly different among the treatments ($P \leq 0.01$). Application of 500 kg/rai leonardite with 100% chemical fertilizers based on soil analysis gave the highest fresh and dry weight of pod per plant. (Figure 4).

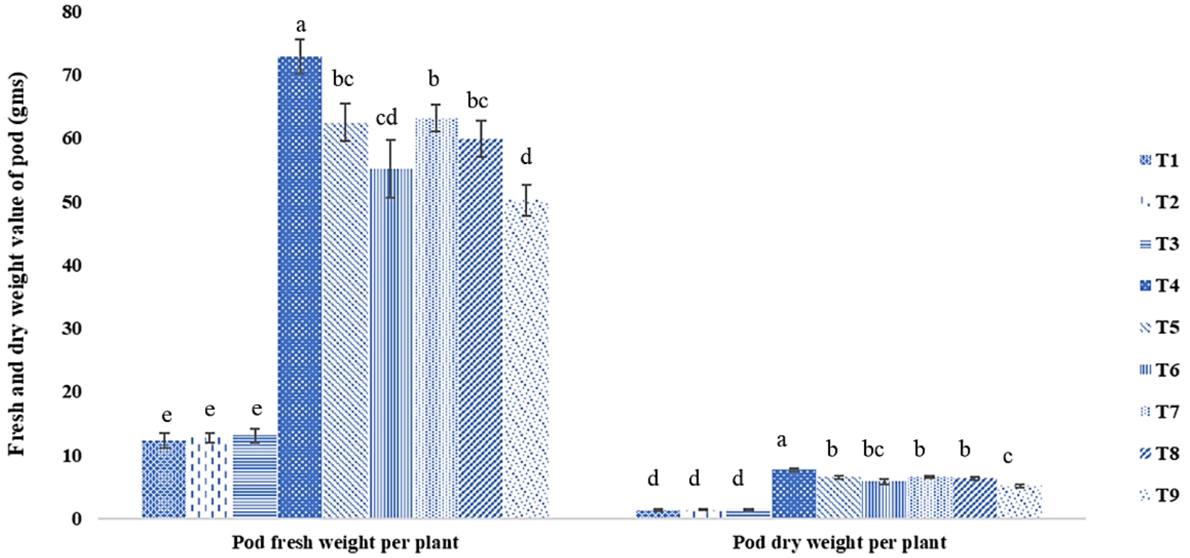

Figure 4. Effect of leonardite and nutrient management on fresh weight and dry weight of pod per plant

The vegetative growth and yield of cowpea treated only with leonardite were lower than those applied with leonardite combined with chemical fertilizers based on soil analysis because leonardite contained low plant nutrient contents (Pochadom et al., 2013). Therefore, leonardite could not replace chemical fertilizers (Totirakul et al., 2009). Rinnarong et al. (2016) reported the vegetative growth and yield of Chinese cabbage in the treatment without leonardite was the lowest among the treatment.

In addition, the vegetative growth and yield significantly increased after leonardite was applied with chemical fertilizers based on soil analysis. The highest plant height, canopy width, stem diameter, leaf width, leaf length, leaf greenness, and fresh and dry weight of pod per plant were received after applying 500 kg/rai leonardite with 100% chemical fertilizers based on soil analysis. The results from this experiment were in agreement with Ngennoy et al. (2014), who reported that humic substances extracted from leonardite mixed with chemical fertilizers enhanced plant nutrient absorption and promoted growth and yield of maize. Sariyildiz (2020) also reported that the most incredible garlic yields were observed when applying leonardite at different doses or the mixture of leonardite with the mineral fertilizer. In addition, leonardite has a high cation exchange capacity (Rittirat, 2017; Pochadom et al., 2013) and has a large amount of organic matter (Totirakul et al., 2009). Soil organic matter enhances water holding capacity and cation exchange capacity, absorption of available nutrients, and increases available plant nutrients and nutrient uptake from soil (Faculty of the Department of Soil Science, 2006; Pompranee, 2017). Sanli et al. (2013) revealed that leonardite could increase available nitrogen, potassium, and phosphorus availability in soil. Thus, applying leonardite and chemical fertilizers based on soil analysis effectively increased the vegetative growth and yield of cowpea.

CONCLUSIONS

The application of 500 kg/rai of leonardite in combination with 100% chemical fertilizer based on soil analysis gave the highest plant height, canopy width, stem diameter, leaf width, leaf length, leaf greenness (SPAD), fresh weight, and dry weight of pod per plant.

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REFERENCES

Benchasri, S., Domyos, S., and Pruthikanee, P. 2014. Potential of yardlong bean production under chemical and organic system in Southern Thailand. Thaksin University, Thailand.

Department of Agricultural Extension. 2019. Cultivation situation of cowpea: 2018-2019. Ministry of Agriculture and Cooperatives, Thailand.

Department of Land Development. 2015. State of soil and land resources of Thailand. The Agricultural Cooperative Federation of Thailand, Thailand.

Enyiukwu, DN., Amadioha, AC., and Ononuju, CC. 2018. Nutritional significance of cowpea leaves for human consumption. Greener Trend in Food Science and Nutrition. 1(1): 1-10.

Faculty of the Department of Soil Science. 2006. Introduction to soil science. Kasetsart University Press, Thailand.

Goncalves, A., Goufo, P., Barros, A., Dominguez-Perles, R., Trindade, H., Rosa. E A S., Ferreira, L., and Rodrigues, M. 2016. Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. Journal of the Science of Food and Agriculture. 96: 2941-2951.

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwang, R., Jayawardana B.C., Nammi, S., and Liyanage, R. 2018. Cowpea: an overview on its nutritional facts and health benefits. Jounal of the Science of Food and Agriculture. 98(13): 4793-4806.

Khan, A.R., Alam, S., Ali, S., Bibi, S., and Khalil I.A. 2007. Dietary fiber profile of food legumes. Sarhad Journal of Agriculture. 23(3): 763-766.

Landrot, S., Armartmontree, C., Khongkaew, C., and Jutamanee, K. 2020. Leonardite-based organic fertilizer production complying with the organic fertilizer standard of the department of agriculture. King Mongkut's Agricultural Journal. 38(1): 93-103.

Ngennoy, S., Jutamanee, K., Rungmekarat, S., and Kaokaew, S. 2014. Effect of humic substance and chemical fertilizer application on plant nutrient, growth and yield of maize (*Zea mays* L.). Proceedings of 52nd Kasetsart University Annual Conference: Plant, 4-7 Feb 2014. Kasetsart University, Bangkok. p.252-259.

Pochadom, S., Khaokaew, S., Sooksamiti, P., Jutamanee, K., and Landrot, G. 2013. Chemical characterizations of leonardite from lignite mine for agricultural application. Proceedings of 51st Kasetsart University Annual Conference: Plant. Kasetsart University, Bangkok. p.243-249.

Pompranee, P. 2017. Soil science. Faculty of Science and Technology, Nakhon Pathom Rajabhat University.

Ratanaprommanee, C., Chinachanta, K., Chaiwan, F., and Shutsrirung, A. 2016. Chemical characterization of leonardite and its potential use as soil conditioner and plant growth enhancement. Asia-Pacific Journal of Science and Technology. 22(4): 1-10.

Rinnarong, S., Chittaladakorn, A., and Thienhirun, P. 2016. Effect of leonardite on growth and yield of Chinese cabbage (*Brassica campestris* var.*chinensis*). The 6th STOU National Research Conference. P.1-13.

Rittirat, A. 2017. Study of the use of the coal power plant by-product (Leonardite) to adsorb. Master Degree of Environmental Engineering, Suranaree University of Technology, Thailand.

Sanli, A., Karadogan, T., and Tonguc, M. 2013. Effects of leonardite applications on yield and some quality parameters of potatoes (*Solanum tuberosum* L.). Turkish Journal of Field Crops. 18(1): 20-26.

Sariyildiz, T. 2020. Effects of leonardite and mineral fertilizer applications on plant growth and soil quality of garlic (*Allium sativum* L.). Turkish Journal of Agriculture-Food Science and Technology. 8(8): 1763-1772

Totirakul, V., Sooksamiti, P., and Tokamolthom, J. 2009. The potential of the leonardite from lignite Mae Mho mine Lampang province for preparation of humic acids and humic salts. The Office of Primary Industries and Mines Region 3, Ministry of Industry, Thailand.

INSTRUCTION FOR AUTHORS

1. Types of articles and formats

Manuscripts may be submitted to JSAT in the form of Review Articles or Research Articles and must be clearly and concisely written in English with appropriate pages.

2. Preparation of manuscripts

All manuscripts must be prepared and submitted as single Word file (.docx) (Word 2007 or higher). For English font, please use "Time New Roman" with text size of 12 point. Please prepare your manuscript in double-space on A4-sized page. Line number must be used continuous numbering in manuscript. The submitted manuscript should be organized as follow:

2.1) Title

The title of manuscript should be supplied with enough information to catch the attention of readers.

2.2) Author names and affiliations

Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Indicate the corresponding author by "*". If authors have different addresses, numbered superscripts are required to refer to each address provided. The format of authors' affiliations should be: 1. Department, University, City name, State name, Postal code and Country. Corresponding author must inform email.

2.3) Abstract and keywords

Abstract - A concise abstract with maximum of 200-250 words identifying the new and significant results of the study must be provided for all manuscripts.
Keywords: – At least 3 to 5 keywords must be given below abstract and use "comma (,)" to separate each.

2.4) Introduction

Author should prepare the introduction with suitable length and keep it as short as possible.

2.5) Materials and methods

This section contains details about materials, techniques, apparatuses, experimental design, and environment. Sufficient detail should be provided to permit the reader to repeat the experiments. For the manuscript reports the utilization of chemical, please provide the purity of all chemicals used in this part.

2.6) Results and discussion

In this part, the results and discussion can be combined or separated depends on author design. Please use tables, graphs, diagrams, and photographs to provide a clear understanding of the results. Quantitative measures of significance (P-values) should be presented. Authors may use either absolute P-values or a defined significance level as long as usage is consistent.
Discussion contains the interpretation of the results into existing literature. It should be clear and concise, address the related mechanisms and their significance.

2.7) Conclusions

Clear and significant conclusions should be provided and related to the results and objectives of your work.

2.8) Acknowledgments

In this section, financial and material support should also be mentioned. Authors should list all funding sources in this acknowledgments section. The names of funding organizations should be written in full. List those individuals who provided support during the research

2.9) References

2.9.1) In text citations, please use name-year system such as:

a. Before texts: Smith (2018)…., Tsuji and Hayate (2012)…., Smith et al.(2019)…., Smith (2018); Tsuji (2012)…., Smith et al.(2018a); Smith et al.(2008b)…..

b. After texts: …. (Smith, 2018), …. (Tsuji and Hayate, 2012), …. (Smith et al., 2019), …. (Smith, 2018; Tsuji, 2012), …. (Smith et al., 2018a; Smith et al., 2008b)

2.9.2) References list

Published literatures or unpublished works must be listed alphabetically by surname of author at the end of the manuscript. Each reference to a periodical publication must include, in order, the name(s) of the author(s), the year of publication, the full title of the article, the publication in which it appears, the volume and inclusive page numbers, and the digital object identifier (DOI), if available. Examples of references are listed as follow:

• Journal article

Halmilton, M.B., Pincus, E.L., Fion, A.D., and Fleischer, R. C. 1999. Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques. 27: 500-507. https://doi.org/xx.xxxxxxxxxx

Gupta, A.P., and Kumar, V. 2007. New emerging trends in synthetic biodegradable polymers-polylactide: A critique. European Polymer Journal. 43: 4053-4074.

• Book

Carr, R.L. 1976. Powder and granule properties and mechanics. Marcel Dekker Publisher, New York.

• Chapter in book

Jackson, M.B. 1982. Ethylene as a growth promoting hormone under flooded conditions. In: Wareing, P.F. (ed) Plant growth substance. Academic Press, London. p.291-301.

• Proceeding, symposia etc.

Pratt, A., Gilkes, R.J., Ward, S.C., and Jasper, D.A. 2000. Variations in the properties of regolith materials affect the performance of tree growth in rehabilitated bauxite mine-pits in the Darling Range, SW-Australia. In: Brion, A., and Bell, R.W. (eds) Proceeding of Remade Land 2000, the International Conference on Remediation and Management of Degraded Lands. Fremantle, 30 Nov-2 Dec 2000. Promaco Conventions, Canning Bridge. p.87-88.

• Dissertation

Senthong, C. 1979. Growth analysis in several peanut cultivars and the effect of peanut root-knot nematode (Meloidogyne arenaria) on peanut yields. Ph.D. Dissertation. University of Florida, Gainesville, Florida, USA.

3. Equation, figure, table and unit of measurement

Equation

All equations presented in the text can be prepared by Equation editor from Microsoft word or Math Type.

Figure

Data presented in figure must be written in English. Color or gray scale figure should be prepared as TIFF format (.tif) with at least 600 dpi. Photograph should be prepared as TIFF (.tif) or JPEG (.jpg) format with at least 600 dpi. Figure with caption should be placed next to the relevant text, rather than at the bottom of the file.

Table

Data presented in table must be written in English. Table with caption and footnote should be placed next to the relevant text, rather than at the bottom of the file.

Unit

Author should use the International System of Units (S.I.)

4. Structure of manuscript

- Title
- Author names and affiliations
- ABSTRACT
- Keywords
- INTRODUCTION
- MATERIALS AND METHODS
- RESULTS AND DISCUSSION (The results and discussion can be combined or separated depends on author design)
- CONCLUSIONS
- ACKNOWLEDGMENTS (if any)
- REFERENCES

5. After acceptance

Authors would be asked to send the “Copyright Transfer Agreement” to JSAT.

6. Publication or processing fee

There is no publication or processing fee.

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