Effect of humic acid extracted from Thailand’s leonardite on rice growth

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Abstract

Humic acid have been successfully extracted from leonardite using base-acid treatment. Humic acid is an essential part of soil. This material, present in good soil, makes available of uptake to the plant and the soil nutrients for improving the physical structure of the soil. To extract humic and fulvic acid, the Leonardite is processed in a strongly basic aqueous solution. To precipitate the humic acid, the solution is adjusted to pH 1 with a mineral acid. Carbon, Oxygen, Nitrogen, Aluminium, Silicon and Potassium were extracted from humic acid in humate form (Humic acid reacted with KOH). Rice grower have expressed interest about using humate as a soil fertilizer. This research, humic acid was used to amend soils to increase organic matter and application on rice berry growth. In addition, humic acid and leonardite was beneficial to leaf and root growth of rice berry compared with the control.

1. Introduction

Humic substances (HSs) are the components of humus which are high molecular weight compounds that together form the brown to black hydrophilic, molecularly flexible, polyelectrolytes called humus [1]. Humic substances are organic compounds that occur everywhere in soils, sediments, water, and landfills [2]. Abundant natural resources of humic substances in Thailand are Leonardite that available in lignite mine from Lampang province, Northern of Thailand [3]. Leonardite is a byproduct of lignite mine. It is natural oxidation product of lignite with brown and coal-like appearance, associated with near surface mining [4]. Humic substance from Leonardite can be divided into three main fractions by solubility in various pH: Humin, Humic acid (HA) and Fulvic acid (FA) [5]. Humic substances can extract from Leonardite, soil and sediments by base-acid treatment process that using strong base and strong acid solution for precipitation of Humin, Humic acid and fulvic acid fraction [6]. Humin is an organic matter that is insoluble in water at all pH. These dark brown solids are inhomogenous and their structures are often vaguely described. HA and FA solution can be extracted form soil and other solid phase using a strong base at high pH. Humic acids are insoluble at low pH, that they can precipitate by adding strong acid or adjust the solution to pH 1. Fulvic acid is the materials that can solute at any base-acid pH but can precipitate by adjust pH to 4-5 [7].

Humic substance is very important for soil agriculture that affects physical and chemical properties and improves soil fertility [8]. Humic acid is the principle component of humic substance extraction because it can use in various applications. Humic acid are known to significantly affect the behavior of pollutants in natural environments, such as trace metal speciation and toxicity [9], used as a natural feedstuff, it has positive effects on the health of various animal species. Humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups. The hypothetical structure for humic acid, shown in Figure 1, contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and COOH groups variously placed on aromatic rings [10].

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Applications of HA also showed the improved plant growth, seed germination, and fruit quality [11]. Humic substances can serve as nitrogen, phosphorus, and sulfur reservoirs; improve soil structure, aeration, and drainage; and increase buffering and exchange capacities by natural buffering group (-COOH) [12]. This research is study extraction of humin and humic acid in leonardite from of lignite Mae Mho Mine from Lampang province in Northern of Thailand by using base-acid treatment and centrifugation. The optimum condition was used in extraction process. The objective of this work is to separate humin and humic acid from leonardite with optimum condition and improve their agricultural application by study effect of humic acid on plant growth. Furthermore, humic acid is used as soil fertilizer for rice berry growth.

2. Experimental

2.1 Materials

Leonardite soil sample was collected from lignite mine at Mae moh, Lampang province, Northern Thailand since August 2016. The sample was dried at 80ºC for 24 h to remove water and moisture contents. After that, the soil piece was milled into powder by mortar and sieved for selection of particle size between 180 to 500 μm.

2.2 Method of humic acid extraction

The Humic substance, extracted from leonardite, had been carried out with the following method, suggested by D. gracia [6], using base-acid treatment. Soil extraction experimental consists of mixing 40 g of dry leonardite powder with 400 ml of 0.1 M KOH during 3 h at room temperature [13]. Then, the soluble, contain HA+FA, was separated from insoluble fraction containing humin by centrifuge at 5000 rpm for 15 min. The humin fraction was dried at 90ºC for 24 h. The HA+FA solution were carefully removed and adjust to pH 2.0 by adding concentrate HCl. The precipitates formed at pH 2.0 that conventionally known as humic acid (HA) were separated from solution by centrifuge at 5000 rpm for 15 min and dried at 90ºC for 24 h.

2.3 Humic acid characterization

Humic substances were characterizing morphology, element composition and chemical structure by Scanning electron microscope (SEM), Energy Dispersive X-Ray Spectrometer (EDX), Fourier transform infrared spectroscopy (FT-IR). In FT-IR spectroscopy, one milligram of dried humic fraction was mixed, ground with 100 mg of potassium bromide (KBr) and then mechanically pressed to form a pellet.

2.4 Plant growth

Riceberry seeds were soaked in water for 2 days and placed inside a Petri dish on a moist paper towel for seedling of Riceberry. The Riceberry seeds with 1 cm. Root were place in nursery tray for seedlings. After 7 days, rice seedlings were moved to plant pots five seedlings per pots. Humic acid 1000 mg/L was treated to Riceberry every 7 days after 30 days with 4 pots per condition. Every 14 days, the lengths of root and shoot and the weights of root and shoot were measured.

3. Results and discussion

3.1 Extraction of humic acid

3.1.1 Element analysis of humic acids

The chemical compositions of leonardite and extracted humic substances were shown in Table.1. Due to humic substances are non-stoichiometric materials they must be characterized in terms of their average properties [14]. On the other hand, humin is soil composition, which separated from leonardite, so their structures have higher aluminosilicate group, oxygen content and few metals than humic acid. Conversely, humic acid has higher carbon content than humin due to their structure is complex aromatic macromolecules with...
Table 1. The composition of leonardite and humic substance derived from leonardsite.

<table>
<thead>
<tr>
<th>Element (%)</th>
<th>leonardite</th>
<th>Humin</th>
<th>Humic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>16.54</td>
<td>11.89</td>
<td>19.53</td>
</tr>
<tr>
<td>O</td>
<td>53.32</td>
<td>49.59</td>
<td>32.85</td>
</tr>
<tr>
<td>Al</td>
<td>9.95</td>
<td>11.13</td>
<td>6.96</td>
</tr>
<tr>
<td>Si</td>
<td>16.73</td>
<td>22.26</td>
<td>11.93</td>
</tr>
<tr>
<td>Cl</td>
<td>0.01</td>
<td>0.01</td>
<td>12.09</td>
</tr>
<tr>
<td>K</td>
<td>1.38</td>
<td>2.94</td>
<td>13.03</td>
</tr>
<tr>
<td>Other metal</td>
<td>2.07</td>
<td>2.19</td>
<td>1.56</td>
</tr>
</tbody>
</table>

organic carbon content [10]. Moreover, the composition of humic acid consist of potassium (K) and Chloride (Cl) because the potassium species in base solution was formed into metal halide salt (KCl) and was supported on humic acid surface [6]. Humic acid with potassium halide salt in solid state general called potassium humate [15].

3.1.2 Morphology and structural of humic substance

SEM images in Figure 2 reported the fractions of humic substances extracted from leonardite with different microstructures morphology. In Figure 2 (a), the morphology of humin is shown which is nonconductive bulk material with few porous structure. Furthermore, in Figure 2 (b) show the microstructure morphology of humic acid. The HA fraction morphology are composed of particles with various shapes and sizes distribution. SEM image of humic acid with potassium halide salt of potassium humate was showed in Figure 3. The result indicates that humic acid from leonardite that extracted with potassium hydroxide observe well-disperse and non-agglomerate of metal halide salt. The potassium salt particle was supported on humic surface and form humic acid into solid state [15].

3.1.3 Structural and spectroscopic characteristics of the humic substances

IR spectra spectrometry was used to characterize structural of humic substances. The FT-IR spectra of leonardite and extracted humic substances are shown in Figure 4. The spectra were recorded in the 4000–400 cm⁻¹ range. The IR spectra bands were similar in various composition of humic substances. In addition, the spectra bands are around 3400–3300 cm⁻¹ (O–H and N–H stretch), 1680-1490 cm⁻¹ (aromatic C=C, C=O, COO⁻), 1120-920 cm⁻¹ (C–OH stretch of aliphatic alcohol) and 720-550 cm⁻¹ (deformation of –COOH). These results accord with those reported by Naidja A. et. Al [16] and indicate humic acid that was extracted from Thailand’s leonardite is purity product with similar spectra with commercial HA [17].

Figure 2. SEM image of (a) Humin and (b) Humic acid 0.01M KOH and particle size at 180 μm for 3h at room temperature.

Figure 3. SEM image of potassium humate.
From IR spectra indicate that humic acid has higher intensity of another functional group than humin and leonardite, respectively. According with peak area result in Table 2 showed the peak area of OH group, C=C group, C–OH group and COOH group from FT-IR spectra of leonardite, humin and humic acid extracted from Thailand’s leonardite. The results indicate humic acid has higher peak area at 1680-1490 cm⁻¹ (aromatic C=C, C=O, COO–), 1120-920 cm⁻¹ (C–OH stretch of aliphatic alcohol) and 720-550 cm⁻¹ (deformation of –COOH) than humin and leonardite. Because of aromatic structure and carboxylic group of humic acid were separated from leonardite that according with Hypothetical molecular structure of humic acid [10]. In contrast, humic acid showed stronger peak area at 3400–3300 cm⁻¹ (O–H stretch) than humin and leonardite that attributed to aromatic and phenolic compounds. From O–H stretch indicates that humic acid contain more aromatic and phenolic compounds than other fractions. From the result, it can conclude that humic acid extracted from leonardite has carboxylic acid functional group and aromatic carbon on their structure and higher than humin and leonardite, but humin and leonardite has higher oxygen content and aluminosilicate group on their structure due to soil composition.

3.2 Effect of potassium humate treated on rice growth

3.2.1 Plantlet height

Potassium humate (K-HA) from leonardite was treated to Riceberry every 7 days after 30 days old plantlet and collected plantlet every 14 days. Figure5 showed a significant difference between control (black bar) and potassium humate (gray bar) effect on rice berry plantlet height. In Figure 5(a) and 5(b), Length of root and shoot in rice plantlets that were collected at 37 days wasn’t significantly change between K-HA and untreated. Conversely, lengths of root and shoot in treated Riceberry was higher than untreated at more than 44 days old plantlet. However, potassium humate applications increased root and shoot lengths compared with untreated ones. The absorption of potassium humate into Riceberry plantlets has a positive influence on plant germination and plant development; resulting in higher plant growth rates.

3.2.2 Fresh weight

Figure 6(a) and 6(b) were shown the effect of potassium humate on weight of Riceberry plantlet. According with results of root and shoot lengths, weights of root and shoot in Riceberry plantlets that were collected at 37 days wasn’t significantly change between K-HA (gray bar) and untreated (black bar). But weights of root and shoot in treated Riceberry were higher than untreated ones at 44 days old plantlet. Therefore, potassium humate uptake has beneficial to biomass production of Riceberry growth. Because of humic substances improve water absorption and stimulate biomass accumulation [18].

Table 2. The peak area of functional group of leonardite and extracted humic substance.

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Composition</th>
<th>leonardite</th>
<th>humin</th>
<th>Humic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>-COOH deform</td>
<td>0.2</td>
<td>0.23</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>-C-OH aliphatic</td>
<td>2.67</td>
<td>2.83</td>
<td>3.34</td>
<td></td>
</tr>
<tr>
<td>C=C, C=O aromatic</td>
<td>0.09</td>
<td>0.65</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>H bond in O-H</td>
<td>0.31</td>
<td>0.31</td>
<td>0.28</td>
<td></td>
</tr>
</tbody>
</table>

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Potassium humate uptake was beneficial to leaf, body and shoot growth of Riceberry compared with the control. While humic substances promote plant growth through improving water uptake, influence the soil’s structure, release of plant nutrients from soft minerals, increased availability of trace minerals, and in general improved soil fertility at indirectly effect [19]. One stimulative effect of humic substances on plant growth is enhanced uptake of major plant nutrients: nitrogen phosphorus and potassium [20]. For directly effect, changes of plant metabolism that occur due to the uptake of organic macromolecules (humic substance)

4. Conclusions

Humic substance can be extracted from Thailand’s leonardite soil by base-acid treatment and centrifugation. Humic acid that extracted from leonardite with optimum condition was shown spectra band of O–H and N–H stretch, aromatic C=C, C=O, COO– and –COOH. The amino group and carboxylic group attached to natural buffering group for the ion exchanged in soil agriculture. The composition of humic acid consist of C, O, Al, Si and KCl. Humic substances was used to condition soils either by applying it directly to the soil as soil fertilizer. This research on the impact of short-term humic acid application on rice berry growth. HAs and leonardite was beneficia to leaf and root growth of rice berry compared with the control.

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