A potentially sustainable weed control method using urease enzymes extracted from weeds

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Summary Weed management and control are essential for successful crop production. In recent years, there has been increased interest in the use of sustainable biological approaches for weed control due to their potential environmental and economic benefits. In this study, the enzyme-induced carbonate precipitation (EICP) approach was adopted to form a soil crust through calcium carbonate bonding using plant-sourced urease enzymes extracted from the weed paddy melon (*Cucumis myriocarpus* Naud.), urea and calcium chloride solution. The penetration and erosion resistance of the EICP-treated soil crust was then measured.

The results from this study show that the EICP-treated soil crust exhibited a significant surface hardening with a maximum penetration resistance of 1307 kPa and significantly high resistance to raindrops and wind erosion compared to untreated soil. The penetration and erosion resistance of the EICP-treated soil crust also increased with an increased number of treatment cycles. The outcome of this study shows that an EICP-approach, using crude enzymes extracted from weeds, can achieve a desirable crust penetration strength that may significantly reduce weed seedling emergence. The technique can also be developed as a potentially sustainable method for weed control for uncultivated land such as roadside shoulders and embankments.

Keywords weed control, paddy melon, EICP, urease enzyme.

INTRODUCTION

Weeds are unwanted plants that grow outside their natural ecosystems where they may be of no positive economic importance (Oerke 2006). In many cases, the presence of weeds on farmlands affects the productivity of the land, crop development and yield. Conventional methods of weed control include manual removal and the use of chemical herbicides (Bajwa et al. 2018; Christoffoleti et al. 2007). However, the use of physical and/or chemical methods of weed control is often undesirable or insufficient in many situations. In recent times, the evolution of herbicide-resistant weed ecotypes across the world has further aggravated the situation (Bajwa et al. 2019). One of the most sustainable approaches for weed control can be by preventing weed seedling emergence through soil crusting. However, this approach has not been studied in the literature.

Nonetheless, the influence of naturally crusted soils on plant seedling emergence has been investigated in several studies (Anzooman et al. 2018; Joshi 1987; Laker and Nortjé 2019; Massingue 2002). The emergence of seedlings from crusted soils depends on the seedling emergence force and the soil crust strength (Anzooman et al. 2018; Sinha and Ghildyal 1979). Most importantly, the mechanical resistance of the soil crust often restricts seedling emergence. If the force exerted by a young seedling immediately after germination is less than the resistance of the soil crust, the seedling remains beneath the crust and does not emerge (Awadhwal and Thierstein 1985). Not only do soil crusts provide a mechanical impedance to seedling emergence, but they also obstruct soil moisture, temperature and gaseous exchange due to their low porosity, thereby limiting the supply of oxygen to germinating seeds. Most studies have reported a negative linear correlation between the percentage of seedling emergence and crust strength with a typical crust strength between 40-700 kPa required to fully hinder seedling emergence (Bennett et al. 1964; Joshi 1987; Massingue 2002; Parker Jr and Taylor 1965; Richards 1953). The variations in the reported threshold of crust strength required for fully hindering seedling emergence as reported in the literature are possibly due to the differences in crop seedlings and the crust strength testing method used in various studies.

Although most studies have investigated the influence of naturally crusted soils on the seedling emergence of crops, none of these has studied the potential of biologically induced crusted soils as a sustainable approach for controlling weed seedling emergence. Therefore, biocementation approaches, such as enzyme-induced carbonate precipitation

(EICP), can be a potentially sustainable means of preventing or reducing weed growth or emergence through natural ground solidification and/or hardening. EICP involves calcium carbonate ($CaCO_3$) precipitation via urea hydrolysis catalysed by plant-sourced urease enzymes (Ahenkorah et al. 2021b). The precipitated $CaCO_3$ forms bonds between the soil particles, which results in ground solidification. In this study, the penetration and erosion resistance of EICP-treated soil crusts was assessed as a potentially sustainable method of weed control for uncultivated lands such as roadside shoulders and embankments. A sustainable source of crude urease enzyme extracted from the weed paddy melon (*Cucumis myriocarpus* Naud.) was used in this study for the treatment of EICP-treated soil crusts.

MATERIALS AND METHODS

Soil type and sample preparation Two soils were used in this study, namely Karoonda silty sand (KSS) and Adelaide industrial sand (AIS). These were both sourced from Adelaide, South Australia. Figures 1(a) and (b) show pictures of KSS and AIS, respectively. The soil samples were mixed with 10% moisture and prepared in round PVC containers (110 mm in diameter and 25 mm in height) to a relative density of ~30%.

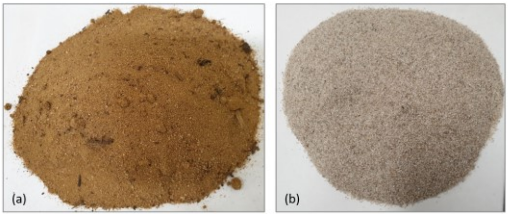

Figure 1. Pictures of different soils used in this study: (a) KSS; and (b) AIS.

Preparation of EICP treatment solution An EICP treatment solution consisting of a mixture of crude urease enzyme solution (extracted from paddy melon seeds) and (equimolar) 0.50 M of cementation solution (containing urea and $CaCl_2$) was prepared. To prepare crude urease enzyme extract, paddy melons were collected from areas surrounding Adelaide, South Australia. Figure 2 shows an image of paddy melon fruits and seeds. A 50 g amount of seeds obtained from the paddy melons were then soaked overnight in 200 mL of deionised water and the solution was homogenised in a blender for approximately 5 minutes. The enzyme-containing solution was then centrifuged twice at ~4400 rpm for 15 minutes and the supernatant was collected as crude urease enzyme extract (Ahenkorah et al. 2021a).

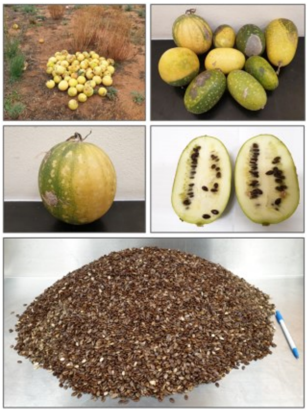

Figure 2. Paddy melon fruits and seeds as a source of plant urease enzyme.

EICP soil treatment process A new EICP treatment approach was developed in this study. Soil samples were treated with only one cycle of EICP treatment. The EICP treatment consisted of spraying 7.5 mL of crude urease enzyme extract from paddy melon seeds and 7.5 mL of cementation solution on top of the specimens from opposite directions at the same time and the sample was then cured at 30 °C for 40-48 hours. This approach allowed the percolation of the treatment solution to the desired depth and prevented concentrated precipitation at the surface or precipitation within the EICP solution before application. The volume of crude urease enzyme and cementation solution used was calculated based on the field capacity of the soil used and a target depth of cementation of ~5 mm. The strength and erosion resistance of the EICP-treated soil crusts were then determined.

Penetration test A handheld penetration test using a Mecmesin AFG500 force gauge fitted with a flat end circular probe of diameter 7-8 mm was used in this study to determine the strength of the EICP-treated soil crust. During the penetration tests, the crust strength at five different locations of the circular surface of the EICP-treated soils, i.e. top (T), bottom (B), left (L), right (R) and center (C) were measured.

Erosion resistance test To evaluate the erodibility of the EICP-treated soil crust, both wind and raindrop erosion resistance tests were conducted in this study. It should be noted that of the two soil types, the AIS was used to prepare samples used for these tests due to its loose and cohesionless nature, making it more susceptible to wind and rainfall erosion. For wind erosion tests, a 1 m long wind tunnel was developed and used in this study. A digital wind speed meter (anemometer) was used to measure the wind speed. Specimens were placed in the middle of the tunnel and were subjected to blowing wind for 1 hour at the set wind speed. The wind speed was adjusted to 10, 20, 30 and 40 km h^{-1} in different tests. The mass of the specimen was measured before and after the test and mass loss was calculated per unit area per unit time. For raindrop erosion tests, deionised water fell from a constant height of 400 mm from the vertical centre of the specimen for 1 hour at various rates (3, 6, 9 and 12 mL min^{-1}). After the test, a vernier calliper was used to measure the erosion cavity diameter at the widest point as well as the erosion cavity depth at the deepest point.

RESULTS AND DISCUSSION

Formation of EICP-treated soil crust By visual inspection, the EICP-treated soils formed a solid crust ~5 mm thick at the surface. The images of each soil before and after EICP treatment are shown in Figures 3(a) and (b) for KSS and 3(c) and (d) for AIS, respectively. Figures 3(e) and (f) show an image of the EICP-treated soil crusts.

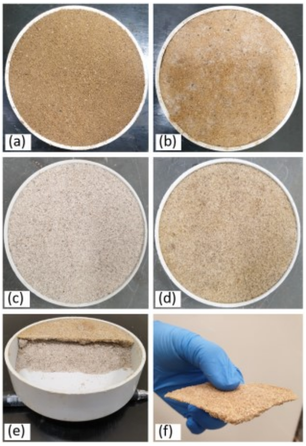

Figure 3. Soil specimen showing: (a) untreated KSS; (b) EICP-treated KSS; (c) untreated AIS; (d) EICP-treated AIS; and (e and f) EICP-treated soil crust.

Strength of EICP-treated soil crust Figure 4 shows the results of crust strength for KSS and AIS measured at five different locations for each sample. The results show that the strength of the crust at the centre of the sample was significantly higher, and indeed was almost twice that of other locations. For example, maximum crust strengths of 1307 kPa and 1050 kPa were achieved at the centre of KSS and AIS, respectively. This could possibly be due to the accumulation of EICP treatment solution at the centre of the sample during the treatment process, leading to high precipitated $CaCO_3$ bonding, resulting in higher strength.

By comparing the crust strength of the two soil types, the KSS soil showed slightly higher strength than the AIS, possibly due to the differences in chemical composition, particle size distribution and particle shape. Overall, both KSS and AIS showed a significantly high crust strength after just one cycle of EICP treatment. This indicates that an EICP-treated soil crust using urease enzyme from paddy melon seeds has enough strength to reduce weed emergence and therefore could be a sustainable approach for weed control, especially for uncultivated lands such as roadside shoulders and embankments.

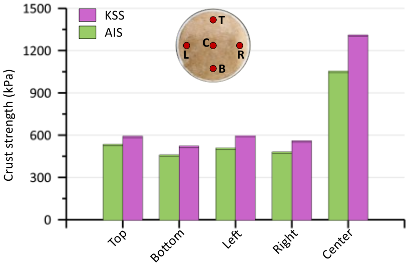

Figure 4. A plot of crust strength at different locations for EICP-treated KSS and AIS.

Erosion resistance of EICP-treated soil crusts Figure 5 shows the mass loss per unit area and unit time during the wind erosion resistance tests. As expected, the mass loss of the untreated AIS increased rapidly with increasing wind speed and reached nearly 42,000 g m^{-2} h^{-1} at a speed of 40 km h^{-1}. The EICP-treated AIS crust showed significantly higher resistance with almost 0 g m^{-2} h^{-1} mass loss up to a speed of 30 km h^{-1}. A relatively small increase in mass loss (~4.5 g m^{-2} h^{-1}) was observed at a speed of 40 km h^{-1}. The results show that the EICP-treated AIS crust exhibited high resistance against wind erosion.

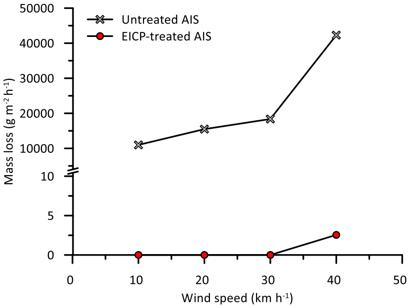

Figure 5. A plot of soil mass loss against wind speed.

Figure 6 shows the erosion cavity radius plotted against the flow rate of rain droplets for untreated and EICP-treated AIS crusts. Overall, the erosion cavity radius increased with an increased flow rate of rain droplets. The untreated AIS showed the largest cavity radius compared to the EICP-treated AIS crust. The high resistance to raindrop erosion exhibited by the EICP-treated AIS crust can be attributed to the presence of $CaCO_3$ bonding within the crust.

Concerning the crusts' erodibility, the EICP-treated AIS crust exhibited higher durability than the untreated AIS crust, and its erosion was less progressive under all conditions.

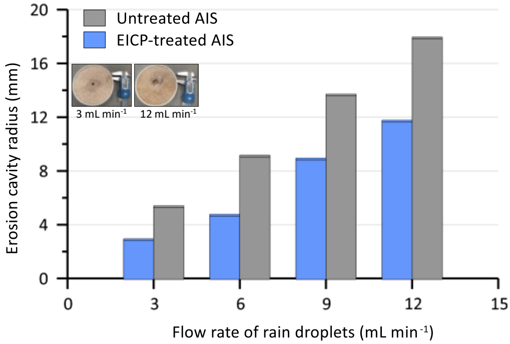

Figure 6. A plot of erosion cavity radius against flow rate of rain droplets.

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REFERENCES

Ahenkorah, I., Rahman, M. M., Karim, M. R., and Beecham, S. (2021a). "Enzyme induced calcium carbonate precipitation and its engineering application: A systematic review and meta-analysis." *Construction and Building Materials*, 308(15), 125000.

Ahenkorah, I., Rahman, M. M., Karim, M. R., Beecham, S., and Saint, C. (2021b). "A review of Enzyme Induced Carbonate Precipitation (EICP): The role of enzyme kinetics." *Sustainable Chemistry*, 2(1), 92-114.

Anzooman, M., Dang, Y. P., Christopher, J., Mumford, M. H., Menzies, N. W., and Kopittke, P. M. (2018). "Greater emergence force and hypocotyl cross sectional area may improve wheat seedling emergence in sodic conditions." *Plant Science*, 277, 188-195.

Awadhwal, N., and Thierstein, G. (1985). "Soil crust and its impact on crop establishment: a review." *Soil Tillage Research*, 5(3), 289-302.

Bajwa, A. A., Khan, M. J., Bhowmik, P. C., Walsh, M., and Chauhan, B. S. (2019). "Sustainable weed management." *Innovations in Sustainable Agriculture*, Springer, 249-286.

Bajwa, A. A., Nguyen, T., Navie, S., O'Donnell, C., and Adkins, S. (2018). "Weed seed spread and its prevention: the role of roadside wash down." *Journal of Environmental Management*, 208, 8-14.

Bennett, O., Ashley, D., and Doss, B. (1964). "Methods of reducing soil crusting to increase cotton seedling emergence." *Agronomy Journal*, 56(2), 162-165.

Christoffoleti, P. J., Carvalho, S., Nicolai, M., Doohan, D., and VanGessel, M. (2007). "Prevention strategies in weed management." *Non-chemical weed management: principles, concepts technology*, CABI, Oxfordshire, 1-16.

Joshi, N. (1987). "Seedling emergence and yield of pearlmillet on naturally crusted arid soils in relation to sowing and cultural methods." *Soil Tillage Research*, 10(2), 103-112.

Laker, M. C., and Nortjé, G. P. (2019). "Review of existing knowledge on soil crusting in South Africa." *Advances in agronomy*, 155, 189-242.

Massingue, F. I. (2002). "The effect of crop residue cover and soil texture on crusting." University of the Free State.

Oerke, E.-C. (2006). "Crop losses to pests." *The Journal of Agricultural Science*, 144(1), 31-43.

Parker Jr, J. J., and Taylor, H. M. (1965). "Soil strength and seedling emergence relations. 1. Soil type, moisture tension, temperature, and planting depth effects." *Agronomy Journal*, 57(3), 289-291.

Richards, L. A. (1953). "Modulus of rupture as an index of crusting of soil." *Soil Science Society of America Journal*, 17(4), 321-323.

Sinha, A., and Ghildyal, B. (1979). "Emergence force of crop seedlings." *Plant Soils Foundations*, 51(1), 153-156.