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Effect of seed pelleting with different matrices on physical characteristics and seed quality of lettuce (*Lactuca sativa*)

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Abstract A suitable type of pelleting material is essential for the successful development of a lettuce seed pelleting formula. Pelleting seeds with calcium sulfate ($CaSO_4$) alone or $CaSO_4$-zeolite resulted in easy pellet formation when carboxymethyl cellulose (CMC) 0.4% (w/w) was used as a binder material. Pelleting seeds with $CaSO_4$-bentonite caused them to be the highest friability. However, pelleting seeds with $CaSO_4$ only, with $CaSO_4$-zeolite, and with $CaSO_4$-pumice resulted in a minor friability of the seeds. When tested under laboratory and greenhouse conditions, it took 21 seconds for the coating of seeds pelleted with $CaSO_4$-zeolite to dissolve in the water, which was considered a slow dissolution, and it did not affect their germination. In addition, when tested in laboratory conditions, seeds pelleted with $CaSO_4$-zeolite had a longer root and shoot lengths compared to the non-pelleted seeds. Therefore, pelleting seeds with $CaSO_4$-zeolite (30 g of $CaSO_4$, 100 g of zeolite) using carboxymethyl cellulose (0.4% w/w aqueous) was the most appropriated formula for 'Red Oak' lettuce seeds.

Keywords: Seed enhancement, Microorganism, Organic seed treatments

Introduction

Lettuce (*Lactuca sativa*) is one of the most economically important vegetables in Thailand. There is a demand for consumption throughout the year, especially during festivals, when the demand tends to increase (Information System of Agriculture Production, 2021). It is also a vegetable with high nutritional value, containing vitamin A, vitamin C, calcium, iron, protein, and carbohydrates, among others. In 2017, 26.04 tons of lettuce seeds were imported, worth 34.68 million baht (Office of Agricultural Economics, 2021). In the lettuce production system, seedling cultivation is a very important step. This is because lettuce seed size is small, about 3-4 mm long (Kangsopa *et al*., 2018). In addition, little food is accumulated in the seeds. When the seeds germinate as seedlings, they usually have low and inconsistent germination and low vigor. Hence, farmers with industrial vegetable farms in Thailand prefer to

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import pelleted lettuce seeds from abroad to use in the cultivation system. However, the price of seeds is high and the production cost is five times higher than buying domestic lettuce seeds (Kangsopa *et al*., 2018).

Given the aforementioned problems, the development of a lettuce seed pelleting formula for use in Thailand is extremely necessary because, in the commercial lettuce production system, lettuce seeds are commonly grown under greenhouse conditions. Therefore, seeds with a regular germination rate are needed to reduce disease incidence in the seedling stage and to reduce the production cost for farmers. Seed pelleting is considered a solution to the problem of the small, flat, or irregular size of lettuce seeds. This technique helps increase the seed size and makes them more evenly shaped and easier to grow (Siri, 2015). A key element for successful seed pelleting depends on the matrix material, binder, and active ingredients. In particular, the matrix material increases the seed size to make it easier to be grown without impeding the absorption of water and air (Taylor, 2003). Some of the commonly used matrix materials are calcium carbonate, limestone, bentonite, zeolite, pumice, gypsum, talc, charcoal, acacia powder, vermiculite, and diatomaceous earth (Porter and Kaerwer, 1974; Taylor and Harman, 1990). However, various types of seed pelleting materials also have different physical properties, so they are suitable for different types of seeds. As a result, to make seed pelleting successful, it is necessary to learn the physical properties of the seeds and their quality after being pelleted to confirm the complete results of seed pelleting formula development (Siri, 2015). Guan *et al*. (2013) developed a bilayer pelleting technique to pellet tobacco seeds where bentonite was the first layer. Then they were sprinkled with talc until the seeds were 1.6–1.8 mm thick in diameter, and the pelleting did not affect their germination. Additionally, Kangsopa *et al*. (2018) found that pelleting ‘Green Oak’ lettuce with $CaCO_3$-gypsum (50 g of $CaCO_3$, 200 g of gypsum) led to easy pellet formation. Moreover, the pelleted seeds had low friability, good solubility in water and the pelleting did not interfere with the seed germination process.

The objective of this study was to find a suitable pelleting material formula to pellet ‘Red Oak’ lettuce seeds and monitor changes in the pelleted seeds’ physical appearance, germination rate, and growth of seedlings. The development of the formula is aimed to improve lettuce cultivation for maximum commercial benefit and create added value for lettuce seeds in Thailand.

Materials and methods

This experiment was conducted at the Seed Technology Laboratory and the greenhouse of the Division of Agronomy, Faculty of Agricultural

Production, Maejo University. Organic 'Red Oak' lettuce seeds used as experimental seeds were cultivated in 2020 by the Learning Center for Organic Vegetable Seed Production, Maejo University. The experiment was conducted between January and June 2021. The details of the experiment are as follows.

Lettuce seeds pelleting

A 0.4% w/w aqueous-carboxymethyl cellulose (CMC, Sigma Aldrich) was prepared for use as the pellet-binding agent (Kangsopa *et al*., 2018). Pelleting was conducted in a Model SKK12 (Seeds Processing Plant, Khon Kaen University, Thailand) rotary drum spinning at 40 rpm. All filler materials were procured from Union Chemical Ltd., Bangkok. Filler materials were added to seeds with a carefully metered application of CMC by pipette to prepare the following seven treatments in 10 g lots: non-pelleted seeds, a monolayer of calcium sulfate ($CaSO_4$), and bilayer treatments of $CaSO_4$ with zeolite, pumice, bentonite, talcum, or diatomaceous earth. For bilayer pellet treatments, layer 1 was $CaSO_4$ while layer 2 filler materials enlarged the pelleted seeds to 3-4 mm in diameter (Table 1). Pelleted seeds were dried a room temperature until the moisture content was reduced to 7%.

Table 1. Composition of experimental pellet matrices and additional treatments used for pelleting lettuce seeds

Treatment	Filler 1 Calcium sulfate (g)	Filler 2* (g)	CMC** (g)
Non-pelleted seeds	0	0	0
$CaSO_4$	100	0	0.4
$CaSO_4$-zeolite	30	100	0.4
$CaSO_4$-pumice	30	100	0.4
$CaSO_4$-bentonite	30	100	0.4
$CaSO_4$-talcum	30	100	0.4
$CaSO_4$-diatomaceous earth	30	100	0.4

All numbers represent the weight of filler applied to 10 g seeds. * Filler 2 is as indicated in the treatment column; ** CMC = aqueous-carboxymethyl cellulose.

Physical tests on pelleted seeds

The lettuce seed pellet formation was focused on the difficulty level of the lettuce seed pellet formation using each type of pelleting material that can adhere and covered the seed hull. A score of 1–5 was used for evaluation, 1 = very difficult, two = difficult, 3 = moderate, 4 = easy, and 5 = very easy (Buakaew and Siri, 2018). To observe the friability of pellets, 100 pelleted seeds were randomly selected, weighed, and put in the tablet friability tester, model 45-2200, at 25 cycles/min for 4 minutes (100 cycles). After that, the seeds were weighed to calculate the percentage of friability. This same testing process was repeated four times (Kangsopa *et al*., 2018). In terms of the water solubility of the pelleted lettuce seeds, ten seeds were randomly selected in each of the four testing replications. After that, the pelleted seeds were soaked in 10 ml of water, one seed at a time. The time set to identify the water solubility duration of the seed was stopped immediately when the pellets started to dissolve. Then the solubility time of the pelleted seeds was recorded. This method was adapted from Anderson *et al*. (1969). To check the acidity and alkalinity of the pelleted seeds, 3 g of selected pelleted seeds in each of the four testing replications were tested by being put in a 50-ml beaker containing 30 ml of water. Then pH values of the pelleted seeds were checked by using a pen-type pH meter tester, model PH-03.

Seed measurement

Seed quality test in the laboratory condition

The seed quality of the 50 pelleted and non-pelleted seeds was tested using the top of paper (TP) method in each of the four replications. They were kept in a transparent plastic box (110 × 110 × 30 mm., length × width × height). Then, the box was placed in the germination incubator at 25 ℃, with 80% of relative humidity, 180 μE light intensity, and with 24-hour lighting. After that, various aspects of seed quality were evaluated as follows.

In terms of radicle emergence, in each of the four replications of all experiment methods, 50 seeds were assessed daily from day 1 and day 3, when the root was at least 2 mm. long. As for the speed of radicle emergence, 50 seeds with roots 2 mm in length were counted daily from day 1 to day 3 in each of the 4 replications of all experiment methods. To test the germination of the seeds germinated as normal seedlings, in each of the four replications, the first count of 50 seedlings was done on day 4 and the final count was done on day 7 to find the germination percentage (ISTA, 2019). As for the speed of germination, 50 seeds germinated as normal seedlings were counted daily, where the first count was done on day 4, and the final count was done on day 7, with four replications. Then, the seeds were calculated for the speed of germination based on the method of AOSA (1983). For the growth of seedlings, in each of the four replications, 10 seedlings were assessed on day 7 after germination. The shoot length of the seedlings was measured from the base of the epicotyl to the tip of the leaf. Their root length was measured from the bottom to the tip of the taproot. The seedling length was measured from the tip of the root to the tip of the leaf.

Seed quality test in the greenhouse condition

Seed germination was done by 50 pelleted and non-pelleted lettuce seeds in the seed pit tray with peat moss used as seed material which were counted for germination in each of the four replications. The first count was done on day 4 after germination, and the final count was done on day 7 (ISTA, 2019). The speed of germination for 50 seeds germinated as normal seedlings was counted daily in each of the four replications; the first count was performed on day 4 after the germination, and the final count was done on day 7. Then, the speed of germination was evaluated using the same method used under laboratory conditions. To observe the emergence, out of all 50 seeds in each of the four replications in all experiment methods, the numbers of seeds with a cotyledon emerging from the planting material were counted on day 1 and day 3 after the germination test started, and the shoot length was assessed at 7 days after germination. The speed of emergence was counted daily from day 1 to day 3 after sowing, with 4 repetitions of 50 seeds each. Then, 10 stems of the seedlings as high as the planting material were cut and measured.

Statistical analysis

The percentage of germination was arcsine-transformed to normalize the data before the statistical analysis. All data were analyzed by one-way ANOVA (Complete Randomized Design), and the difference between the treatments was tested by Duncan's Multiple Range Test (DMRT).

Results

The physical appearance of pelleted seeds

Pelleting seeds with a monolayer of calcium sulfate ($CaSO_4$), and pelleting seeds with $CaSO_4$-zeolite resulted in easy pellet formation compared to other seed pelleting methods. $CaSO_4$ alone, $CaSO_4$-zeolite, and $CaSO_4$-pumice had a smooth external surface for the seed coating (Figure 1). $CaSO_4$-bentonite, $CaSO_4$-talcum, and $CaSO_4$-diatomaceous earth had rough, not smooth surfaces. Pelleting seeds with $CaSO_4$-talcum led to 100% friability. However, seeds pelleted with $CaSO_4$ alone, $CaSO_4$-zeolite, and $CaSO_4$-pumice had a lower percentage of friability compared to other pelleting methods: 4%, 3%, and 4%, respectively. At the same time, seeds pelleted with $CaSO_4$-diatomaceous earth dissolved in 2 seconds, and those pelleted with $CaSO_4$ dissolved in 7 seconds. The dissolution was considered very fast and was statistically different compared to other pelleting methods. The pellets of the seeds pelleted with $CaSO_4$-zeolite dissolved in 21 seconds, which was considered to be slower than the pellets of those pelleted with other methods. The pH value of the pelleting material turned out that $CaSO_4$-talcum's pH value was 8.04, which was higher than other pelleting materials. The pH values of the other pelleting materials were between 7.08 and 7.78 (Table 2).

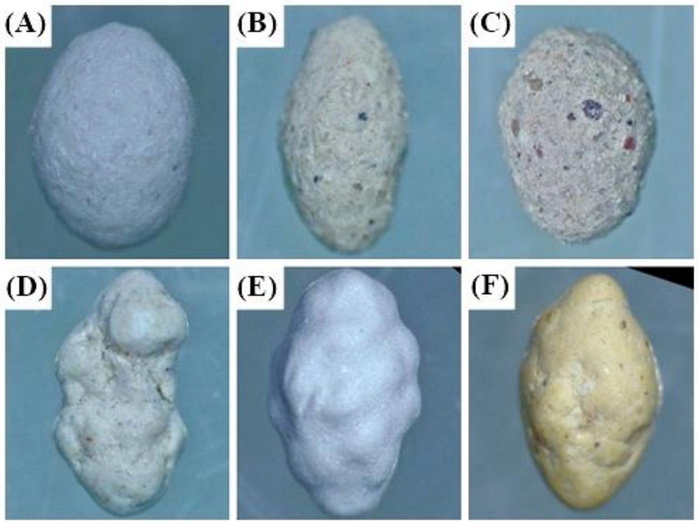

Figure 1. Physical appearance of lettuce seeds pelleted with difference matrices: (A) $CaSO_4$ alone; (B) $CaSO_4$-zeolite; (C) $CaSO_4$-pumice; (D) $CaSO_4$-bentonite; (E) $CaSO_4$-talcum, (F) $CaSO_4$-diatomaceous earth

Table 2. Physical properties of lettuce seeds pelleted with different matrices

Treatment	Forming	Friability of pelleted seed (%)	Dissolution period of pelleted seed (sec)	pH of pelleted seed
$CaSO_4$	4 [1/]	4 d [2/3/]	7 e	7.78 b
$CaSO_4$-zeolite	4	3 d	21 a	7.08 c
$CaSO_4$-pumice	3	4 d	13 c	7.12 b
$CaSO_4$-bentonite	1	65 b	14 b	7.71 b
$CaSO_4$-talcum	3	100 a	11 d	8.04 a
$CaSO_4$-diatomaceous earth	3	28 c	2 f	7.10 c
F-test	-	**	**	**
CV.(%)	-	23.43	67.1	3.58

**: Significantly different at $P \leq 0.01$.

[1/] The forming scores for the seed pelleting: 1 = very difficult, 2 = difficult, 3 = moderate, 4 = easy and 5 = very easy.

[2/] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

[3/] Data are transformed by the arcsine before statistical analysis.

Effect of pelleting lettuce seeds on seed quality

Under laboratory conditions, there was no statistically significant difference between radicle emergence and germination percentage of seeds pelleted with different types and concentration levels of matrix materials (Figure 2A, B). However, non-pelleted seeds had a higher speed of radicle emergence when compared to seeds pelleted with other types of matrix materials except $CaSO_4$ alone and $CaSO_4$-diatomaceous earth (Figure 2C). Seeds pelleted with $CaSO_4$-talcum had a higher speed of germination than seeds pelleted with other materials except with $CaSO_4$ alone, $CaSO_4$-zeolite, $CaSO_4$-pumice, and $CaSO_4$-diatomaceous earth (Figure 2D).

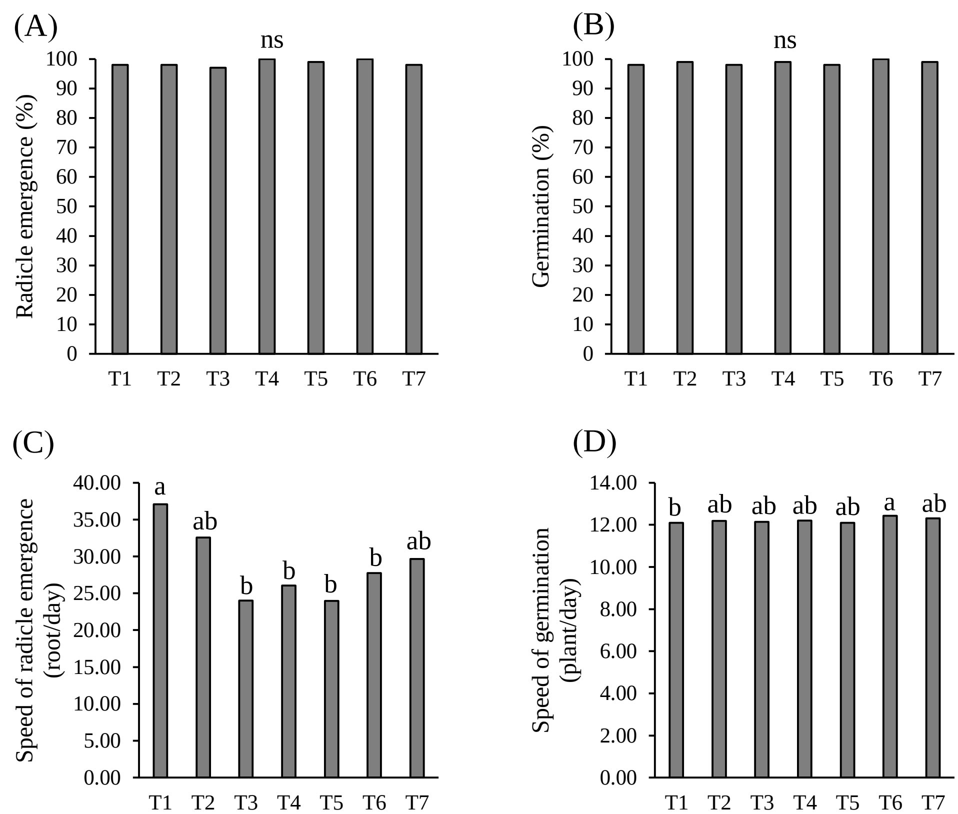

Figure 2. Radicle emergence (A), germination percentage (B), speed of radicle emergence (C), and speed of germination (D) of lettuce seeds after pelleting with different matrices tested under laboratory conditions: T1 = non-pelleted seed, T2 = $CaSO_4$ alone, T3 = $CaSO_4$-zeolite, T4 = $CaSO_4$-pumice, T5 = $CaSO_4$-bentonite, T6 = $CaSO_4$-talcum, T7 = $CaSO_4$-diatomaceous earth

Under greenhouse conditions, non-pelleted seeds had higher emergence than seeds pelleted with other materials, but this was not statistically different from seeds pelleted with $CaSO_4$-talcum (Figure 3A). In addition, the non-pelleted seeds had a higher speed of emergence than those pelleted with all other matrix materials (Figure 3C). Both the percentages of germination and speed of germination of non-pelleted seeds and seeds pelleted with all pelleting methods were not statistically different (Figure 3B, D).

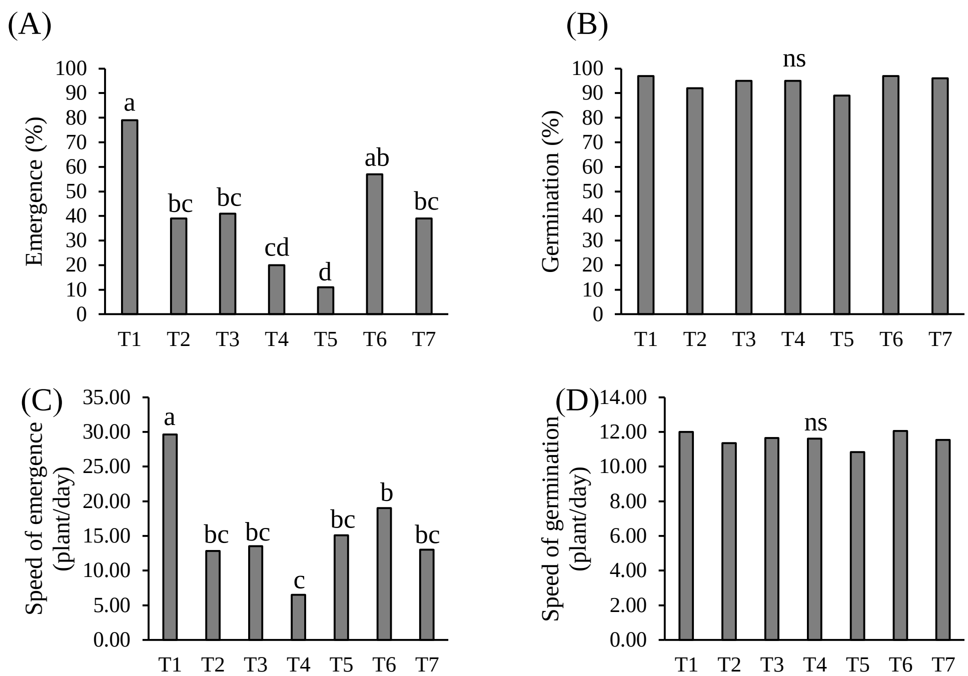

Figure 3. Emergence (A), speed of emergence (B), germination percentage (C) and speed of germination (D) of lettuce seeds after pelleting with different matrices tested under laboratory conditions: T1 = non-pelleted seed, T2 = $CaSO_4$ alone, T3 = $CaSO_4$-zeolite, T4 = $CaSO_4$-pumice, T5 = $CaSO_4$-bentonite, T6 = $CaSO_4$-talcum, T7 = $CaSO_4$-diatomaceous earth

Effect of pelleting lettuce seeds on seedling growth

Under laboratory conditions, the shoot lengths of seeds pelleted with $CaSO_4$-zeolite and $CaSO_4$-pumice was 1.25 and 1.26 cm respectively, which were longer than those pelleted with other matrix materials but not statistically different from seeds pelleted with $CaSO_4$-talcum and $CaSO_4$-diatomaceous earth. At the same time, the root lengths of seeds pelleted with $CaSO_4$-zeolite and $CaSO_4$-diatomaceous earth was 6.81 and 6.89 cm respectively, which were longer than those pelleted with the other methods, but there was no difference when compared with seeds pelleted with $CaSO_4$-pumice. Figure 4 shows that seeds pelleted with all types and concentration levels of matrix materials had longer roots than non-pelleted seeds, especially the seeds pelleted with $CaSO_4$-zeolite and $CaSO_4$-diatomaceous earth. Regarding the seedling length, seeds pelleted with $CaSO_4$-zeolite, $CaSO_4$-pumice, and $CaSO_4$-diatomaceous earth had higher seedling lengths that were statistically different compared to seeds pelleted with other types of matrix materials (Table 3).

Under greenhouse conditions, it was found that seeds pelleted with $CaSO_4$-talcum and $CaSO_4$-zeolite had higher shoot lengths that were statistically different when compared to non-pelleted seeds and those pelleted with other types of matrix materials (Table 3).

Table 3. Shoot, root, and seedling length of pelleted lettuce seed after tested under laboratory and greenhouse conditions

Treatment	**Laboratory**			**Greenhouse**
	Shoot length (cm)	**Root length (cm)**	**Seedling length (cm)**	**Shoot length (cm)**
Non-pelleted	0.97 c [1/]	4.14 e	5.11 d	1.01 bc
$CaSO_4$	1.02 c	5.18 d	6.19 c	0.95 c
$CaSO_4$-zeolite	1.25 a	6.81 a	8.06 a	1.09 a
$CaSO_4$-pumice	1.26 a	6.68 ab	7.94 a	1.01 bc
$CaSO_4$-bentonite	1.17 b	6.14 c	7.31 b	0.64 d
$CaSO_4$-talcum	1.21 ab	6.18 bc	7.39 b	1.11 a
$CaSO_4$-diatomaceous earth	1.23 ab	6.89 a	8.11 a	1.00 bc
F-test	**	**	**	**
CV.(%)	4.63	5.81	4.73	21.6

**: Significantly different at $P \leq 0.01$.

[1/] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

Figure 4. The seedling growth of lettuce was examined under laboratory conditions 7 days after planting. T1 = non-pelleted seed, T2 = $CaSO_4$ alone, T3 = $CaSO_4$-zeolite, T4 = $CaSO_4$-pumice, T5 = $CaSO_4$-bentonite, T6 = $CaSO_4$-talcum, T7 = $CaSO_4$-diatomaceous earth

Discussion

Lettuce seeds are of high value, but their size and shape are still an impediment to cultivation. Therefore, seed pelleting is one of the methods used to improve seed size and shape (Afzal *et al*., 2020). This method will benefit the seed industry and lettuce farmers in Thailand. However, the seed pelleting process is complicated because the seeds' ability to absorb water and oxygen in the pellets affects the seed quality after being pelleted (Siri, 2015). The main goal of this experiment was to identify the best matrix material among the 6 types of materials that are approximately 3-4 mm thick in diameter, oval, and smooth. In the pelleting process, the challenges lie in pellet formation and the combination of matrix, binder, and seeds. Selecting the appropriate matrix material for seeds is also a difficult part of the seed pelleting process. Based on the experiment with various matrix materials, it is easy to form a smooth pellet using $CaSO_4$ alone. Forming the pellet using $CaSO_4$ as a monolayer followed by zeolite and pumice shows that the pelleting material can easily combine with the binder, which results in a bigger pellet that changes according to the shape of the lettuce seeds. Although the seed coatings were formed first with $CaSO_4$ followed by bentonite, they were in an incomplete shape, unhealthy, and had a rough surface.

The friability test of the pellets indicates the potential packing and transport efficiency of the seeds (Siri, 2015). This test is based on the standard practice guidelines for friability testing of tablets in the pharmaceutical industry (Kangsopa *et al*., 2018). Pelleting seeds with $CaSO_4$ alone, or with $CaSO_4$-zeolite and $CaSO_4$-pumice, showed little friability of the pellets. Seeds pelleted with $CaSO_4$-bentonite and $CaSO_4$-talcum had high friability. The results show that it is difficult to form a pellet since it does not retain its shape, breaks easily, and the matrix material does not stick together. Both formulae are therefore not suitable for packing seeds for transport in the industry.

Lettuce seeds need light in the germination process since they cannot germinate well in dark conditions (Nabors *et al*., 1974; Contreras, 2008). Water solubility testing is therefore important for the development of a seed pelleting formula. A good pelleting material must be soluble in water in a reasonable time, which makes it good for promoting water and air absorption, an important factor in the seed germination process. Cultivation of seedlings in the industrial system in Thailand still requires human labor. Handling pellets by hands or forceps may cause moisture on the pellets. If the pellets are exposed to moisture, they can break easily, and that means that the formula used to form the pellet is inappropriate. Seeds pelleted with $CaSO_4$ alone and with $CaSO_4$-diatomaceous earth showed that the pellets dissolved too quickly. On the contrary, results from pelleting seeds with $CaSO_4$-zeolite show that it is the most appropriate formula.

A good seed pelleting formula with the appropriate physical characteristics must not interfere with the seed germination process. All seed pelleting formulas showed no effect on seed germination when tested in both laboratory and greenhouse conditions. The result, however, is in contrast to the report of Buakaew and Siri (2018), who claim that lettuce seeds pelleted with 50 g of calcium carbonate and 300 g of zeolite showed a decrease in germination by 11%. It is possible that in the experiment, the amount of the matrix material in the second layer being reduced from 300 g to 100 g, resulting in no effect on seed germination. In addition, all bilayer pelleting methods used with seeds caused them to have longer shoot, root, and seedling lengths and differ from non-pelleted seeds and those pelleted with $CaSO_4$ alone. During the period of 1 to 3 days in the experiment, the seeds were able to germinate indifferently.

Nevertheless, when pelleting seeds with $CaSO_4$-zeolite, $CaSO_4$-pumice, $CaSO_4$-bentonite, and $CaSO_4$-talcum, there was a relationship between the pellet solubility duration and speed of radicle emergence when tested in the laboratory conditions. However, when considering the emergence above the soil under greenhouse conditions, pelleted seedlings emerged from 1 to 3 days, which is slightly later than germination of the non-pelleted seeds. However, after 4 to 7 days, it was found that all seeds pelleted with all methods showed no difference in germination speed compared to the non-pelleted seeds. Therefore, the seed pelleting formula affects physical characteristics of pellets such as hardness, friability, and water solubility, which affect the germination and growth of lettuce seedlings. In addition, the results obtained from this experiment will be used as data to develop a seed pelleting formula with microorganisms that promote plant growth to improve germination as well as the shoot and root length of seedlings (Haas and Defago, 2005). Moreover, the monitoring of the viability of microorganisms that can attach to the pellets will be the main focus.

When considering the physical properties of pelleted seeds tested under the laboratory and greenhouse conditions, the best matrix material for ‘Red Oak lettuce seeds was $CaSO_4$-zeolite (30 g of $CaSO_4$, 100 g of zeolite) with the use of carboxymethyl cellulose (0.4% w/w aqueous).

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