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Original Article

A new alternative mono-layer matrix for pelleting cucumber (*Cucumis sativus*) seeds

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Abstract

Cucumber seeds are small, oblong, and flat, usually measuring 3–4 mm in length. However, their susceptibility to deterioration and sensitivity to poor storage conditions can result in decreased germination rates and reduced overall vigor. Nevertheless, seed pelleting can effectively shield seeds from harsh environmental factors and slow down their deterioration, thereby preserving the seed quality. The objective of this study was to develop a matrix for pelleting cucumber seeds. The percent germination and radical emergence, as well as other important parameters of the experimental pellets were compared to not pelleted cucumber seeds in laboratory and greenhouse conditions. The treatments in this experiment involved a mono-layer of pumice, talcum, bentonite, calcium sulphate, calcium carbonate, or zeolite, used as a matrix for pelleted seed and bound with methylhydroxyethyl cellulose (MHEC). Seeds treated with talcum or bentonite did not result in smooth and fragile pellets, whereas those treated with $CaSO_4$ exhibited low levels of friability (3.38%) and pellet dissolution (5.11 seconds). Seeds pelleted with $CaSO_4$ showed significantly different germination under laboratory and greenhouse conditions compared to other matrices and to not pelleted seed. This indicates that $CaSO_4$ was suitable for pelleting 'YokKaow' variety of cucumber seed.

Keywords: calcium sulphate, methylhydroxyethyl cellulose, *Cucumis sativus*, cucumber seeds pelleting

1. Introduction

Currently, seed pelleting is an essential technology used in the cultivation of field crops, and in horticulture including flowering plants. Many crops require precision cultivation to reduce the production costs and redundant management. However, mostly seed pelleting is a trade secret. It is important to find a formulation that is suitable for the plant species so that it does not interfere with the germination process. Seed pelleting is the addition of inert materials, such as binders and fine powders, to vary the size, shape, and weight of the seeds (Kangsopa, Hynes, & Siri, 2018; Pedrini, Merritt, Stevens, & Dixon, 2017; Taylor, 2003; Taylor & Harman, 1990). The pellets can be 50-fold increased in size from the original seeds. The pelleting material must possess several qualities. It should be readily available, cost-effective, compatible with conventional binders, allow for unhindered root emergence, have water absorption capabilities, and break up effortlessly (Taylor, 2003). Pellet materials used for pelleting include, for example, calcium carbonate, limestone, bentonite, zeolite, pumice, gypsum, talc, charcoal, acacia powder, vermiculite, and diatomaceous earth (Porter & Kaerwer, 1974; Taylor & Harman, 1990). Examples include lettuce seeds pelleted with calcium carbonate-gypsum and calcium sulphate (Kangsopa *et al*., 2018) or pumice (Jeephet, Atnaseo, Hermhuk, & Kangsopa, 2022); and carrot seeds pelleted with calcium sulphate (Kangsopa, Jeephet, Singsopha, Thawong, & Chantain, 2021).

The seeds of regular cucumbers are easy to cultivate since they are large, measuring approximately 8–10 mm in length. In contrast, the 'YokKaow' cucumber variety, developed in Thailand and widely used in Thai cuisine, has smaller seeds that are approximately 3–4 mm in length. These seeds are thin and light, having a low reserve of nutrition for growth, which makes them challenging to germinate. Moreover, cucumber seeds that have been stored for some time are prone to quality deterioration and seed coat breakdown, resulting in problems for growers (Khaldun &

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Haque, 2009; Powell & Matthews, 2005; TeKrony, 2003). To address these issues, seed pelleting techniques are crucial to compensate for poor seed coat thickness, to delay deterioration, and to improve germination. Additionally, seed pelleting can also be used to incorporate essential substances, such as plant nutrients, biological agents, and pesticides, to enhance seed germination (Contreras, Bennett, & Tay, 2008; Wurr & Fellows, 1985).

Pelleting seeds of the YokKaow cucumber variety can be challenging due to their small size and smooth surface that resists adhesion. The materials for pelleting seeds must also not hinder their ability to germinate. To guide our selection, we followed the guidelines proposed by Kangsopa *et al*. (2018), who evaluated various pelleting materials and their properties, such as particle size, water absorption, adhesion, and toxicity, to identify the most suitable substrates available in Thailand.

Commercial pelleted cucumber seeds have not yet appeared**,** making a new pelleting formulation essential. This is one approach to enhancing high-quality seeds. The goal of this research was to develop a matrix for pelleting cucumber seeds. Six pelleting matrices were applied to cucumber seeds to assess the physical characteristics hardness, friability, and water solubility. Laboratory and greenhouse studies were carried out to compare the germination and seedling growth of seeds in experimental pellets with those of the not pelleted cucumber seeds.

2. Materials and Methods

2.1 Cucumber seed pelleting

Seeds of the hybrid cucumber variety YokKaow (Lion Seeds®) had specific characteristics determined before the experimentation, including a germination rate of 55%, a moisture content of 7%, and a seed length of approximately 3–4 mm. Six types of pelleting materials were used in this experiment: pumice, talcum, bentonite, calcium sulphate, calcium carbonate, and zeolite. Pumice, a light and porous volcanic material, was found to absorb water well. Talcum had fine particles and good fluidity. Bentonite, a type of clay composed mainly of montmorillonite, was also evaluated. We also tested calcium sulphate, a soft sulphate mineral, and calcium carbonate, a common substance found in rocks. Finally, we investigated zeolite, a porous mineral that is also an aluminosilicate and a product of volcanoes. An 0.3% w/w aqueous-methylhydroxyethyl cellulose (MHEC) (Kingsun Chemical) solution was prepared for use as the pellet binding agent. Pelleting was conducted in a rotary drum, Model SKK12, spinning at 40 rpm (Kangsopa *et al*., 2018). Filler materials were added to seeds with carefully measured application of MHEC by pipette to prepare the following six treatments in 10 g lots: not pelleted seeds; layer treatments with pumice, talcum, bentonite, calcium sulphate ($CaSO_4$), calcium carbonate ($CaCO_3$), and zeolite (Table 1). After pelleting with a mono-layer in each treatment, the seeds were approximately 1–1.5 mm in diameter. The pelleted seeds were then dried in a forced air dryer at 35°C until the moisture content reached 7–8%.

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

Treatment	Filler[1] (g)	MHEC[2] (g)
Not pelleted seed	0	0
Pumice	50	0.3
Talc	50	0.3
Bentonite	50	0.3
$CaSO_4$	50	0.3
$CaCO_3$	50	0.3
Zeolite	50	0.3

All numbers represent weight of filler applied to 10 g of seed.
[1] Filler type is as indicated in the treatment column.
[2] MHEC = aqueous-methylhydroxyethyl cellulose used as binder

2.2 Physical tests of pelleted seeds

The formation of cucumber seed pellets during the pelleting process was observed to assess the level of difficulty for each pelleting material in adhering to and covering the seed husks for pellet formation. This was accomplished by assigning a score of 1–5, with 1 indicating a very difficult process, 2 indicating a difficult process, 3 indicating a moderate process, 4 indicating an easy process, and 5 indicating a very easy process. To rate the dissolution period of the pelleted seeds, 10 ml of water was added to a 50 ml cylindrical glass container. The dissolution period was determined as the mean of 10 pelleted seeds per treatment and was concluded when the pelleted seeds cracked and split (Anderson, Conway, Pfeifer, & Griffin, 1970). The friability of pelleted seeds was assessed on 50 pelleted seeds per treatment using a Pharma friabilator tester at 25 ± 1 rpm for minutes (Kangsopa *et al*., 2018).

2.3 Germination testing without and with accelerated aging

In laboratory tests, 50 pelleted or not pelleted seeds were tested using the between paper (BP) method in each of the four replications. A sample from each treatment was placed in the germination incubator at 25°C, with 80% relative humidity, 180 µE light intensity, and 24-hour lighting. There were four replicates per treatment. Testing protocols followed those described by the International Seed Testing Association (ISTA, 2020). Initial germination counts were carried out at 4 and 8 days after incubation, followed by daily germination recordings. Radicle emergence was counted as 2 mm-long radicles 72 hours after sowing, according to ISTA (2020). The germination rate, emergence rate (seedlings emerged/day), shoot length, and root length were determined. The mean germination time was calculated according to Ellis & Roberts (1981).

In greenhouse tests, 50 pelleted seeds per treatment were sown in peat moss (Klasmann-Deilmann GmbH, Ltd., Germany) in 340 × 340 × 60 mm seed trays. Germination counts and shoot length data were collected, as described for the laboratory tests. To examine the emergence, 50 seeds were selected for each method and planted in 4 replications. The cotyledons were observed from the planting material and evaluated on the 3rd day of the germination test.

2.4 Accelerated ageing test

Accelerated ageing of all treatments, including both pelleted and not pelleted seeds, was conducted in a humidity chamber with the following conditions: 100% relative humidity (RH) and 40°C for 48 hours. Laboratory and greenhouse tests on the aged pelleted seeds were conducted as described above.

2.5 Statistical analysis

All experiments were conducted with four replications per treatment. The germination rate was arcsine transformed to normalise the data before statistical analyses were carried out. Laboratory and greenhouse treatments were arranged in a complete randomised plot design (CRD)**,** and the data collected were analysed by one-way ANOVA. The differences between treatments were tested using Duncan's multiple range test (DMRT).

3. Results and Discussion

3.1 Physical appearance of pelleted seeds

Except for talcum and bentonite, which resulted in uneven and rough surfaces (Figures 1B and C), all the four treatments produced smooth edges that followed the shape of the cucumber seeds. The talcum did not adhere to the surface of the cucumber seeds and broke easily. Pumice, talcum, and $CaSO_4$ formed the easiest (Figures 1A, B, and D), followed by $CaCO_3$ and zeolite (Figures 1E and F). Bentonite was more difficult as the pelleting matrix and binder, and the cucumber seed pellets were not homogeneous (Table 2). Pelleting cucumber seeds significantly increased the final product weight. The 100 seed pellet weight per seed ranged from 1.92 (pumice) to 2.62 ($CaSO_4$) fold that of the not pelleted seeds. The pellets made with pumice, $CaSO_4$, $CaCO_3$, and zeolite had one cucumber seed per pellet.

$CaSO_4$ pellets had a dissolution period of 5.11 seconds, while the other pelleting matrices took between 1.27 and 2.81 seconds. Solubility is an important parameter that must be tested. Because cucumber seed pelleting adds a thin layer of pelleting material to the seed coat, it may cause the covering to dissolve too easily when exposed to slight moisture from external factors. $CaSO_4$ was shown to take 5.11 seconds to split. Other pelleting materials with a solubility of 1.27–2.81 seconds had too quick solubility. The experimental results demonstrated that all the seed pelleting treatments led to rapid dissolution of the pellet. In the case of cucumber seed pelleting, this involves coating the seed with a pelleting material similar to the film coating technique. This technique is similar to the seed encrusting method but with a slightly thicker layer. Moreover, cucumber seeds are already relatively large compared to seeds of tobacco, lettuce, and carrot, among others, so there is no need to increase their size by as much as 30–40 times. For these reasons, the layer of the pelleting matrix on the cucumber pelleted seeds dissolves faster than that of smaller seed varieties. In addition, Kangsopa *et al.* (2018) reported that lettuce seed pelleting with the first layer of calcium carbonate, followed by a second layer of bentonite, pumice, or gypsum, had 94, 29, or 35 seconds water solubility, respectively. However, the solubility time in the current study was between 1 and 5 seconds and therefore did not impede the absorption of moisture or other seed germination factors. Cultivators require seed consistency, fast germination, and the absence of disease in the seedling stage.

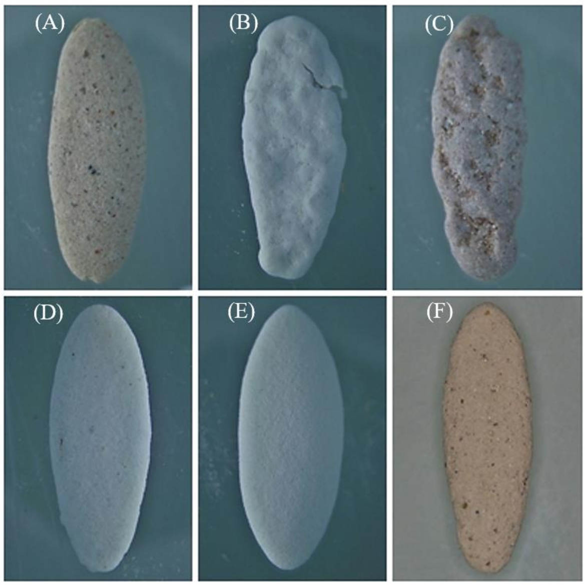

Figure 1. Physical appearances of cucumber seeds pelleted with alternative matrices (10x magnification): (A) pumice; (B) talcum; (C) bentonite; (D) calcium sulfate; (E) calcium carbonate; and (F) zeolite

Table 2. Physical properties of cucumber seeds pelleted with alternative pelleting materials

Treatment	Forming	Seeds/pellet	Pellet 100-seeds weight (g)	Pellet dissolution (seconds)	Friability (%)
Not pelleted	-	-	2.31 g	-	-
Pumice	5	1.00 b	4.43 f	2.81 b	11.46 c
Talcum	5	1.25 a	5.75 b	2.30 b	64.54 a
Bentonite	1	1.50 a	5.00 d	1.70 b	37.96 b
$CaSO_4$	5	1.00 b	6.04 a	5.11 a	3.38 d
$CaCO_3$	4	1.00 b	5.47 c	1.27 b	62.87 a
Zeolite	4	1.00 b	4.58 e	1.62 b	8.74 d
F-test	-	**	**	**	**
CV. (%)	-	7.71	2.21	2.73	8.91

** Interactions significantly different at $P \leq 0.01$. Means in one column with different letters are significantly different ($P \leq 0.05$). Coefficient of variation, CV (%), is the percentage variation in the mean across treatments.

Friability testing has been applied by the pharmaceutical industry in physical quality testing. This parameter is important for the seed pelleting industry. This is an indicator of packaging and transportation potential (Jeephet *et al*., 2022). Pellet matrices of talcum, $CaSO_4$, and bentonite were highly friable compared to the other treatments (Table 2). This indicates that $CaSO_4$ is a more suitable pelleting material in combination with cucumber seeds than the other types of pelleting materials.

3.2 Germination and seedling growth

Under laboratory conditions, all the pellet matrices had significantly higher germination percentages than the not pelleted seeds (Figure 2A). Zeolite pellets had less radicle emergence than the other pelleting matrices (Figure 2C). All these pelleting methods showed significantly higher root lengths than for the not pelleted seeds (Figure 3); however, bentonite and $CaSO_4$ had significantly higher shoot lengths than the not pelleted seeds (Table 3). These pelleting matrices, when coated on seeds, showed that they did not interfere with the germination process; in contrast, the seed germination was greater than that of the not pelleted seeds. Jeephet *et al.* (2022) showed that the seed pelleting bilayer matrices of $CaSO_4$-zeolite, $CaSO_4$-pumice, $CaSO_4$-bentonite, $CaSO_4$-talcum, and $CaSO_4$-diatomaceous earth did not impede the germination of lettuce seeds. In addition, the pelleting material can be composed of various plant nutrients, such as silica, sulphur, and calcium (Kathpalia & Bhatla, 2018). These are clearly more effective in terms of root and shoot length than the not pelleted seeds.

Under greenhouse conditions, $CaSO_4$ gave a significantly higher germination percentage (Figure 2B), emergence rate (Figure 2D), and shoot length compared to the other experimental pellet matrices (Table 3). $CaSO_4$ pellets showed the highest germination because of the slight friability and the slow absorption of water. In addition, the $CaSO_4$ pellet matrix was shown to promote seedling growth more than the other treatments. Calcium sulphate is used in agricultural and horticultural fertilisers, dressings, and pesticides. It provides a natural source of calcium and sulphur, which can be directly assimilated by plants and are vital to fertilisation and healthy plant growth (Saint-Gobain Formula, 2009).

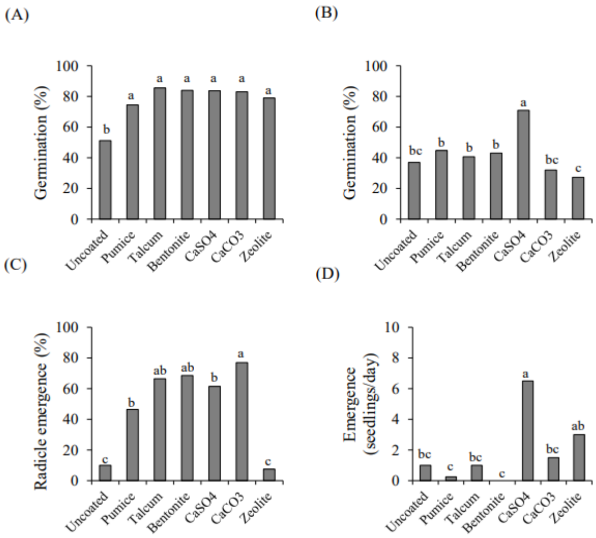

Figure 2. Germination and emergence (■) of pelleted cucumber seeds in the laboratory (A and C) and in a greenhouse (B and D)

Figure 3. Effects of alternative matrices for pelleting cucumber seeds on radicle emergence 72 hours after planting tested under laboratory conditions. (A) Not pelleted seed; (B) pumice; (C) talcum; (D) bentonite; (E) calcium sulfate; (F) calcium carbonate; and (G) zeolite

Table 3. Root length, shoot length and mean germination time of pelleted cucumber seeds under laboratory and greenhouse conditions

Treatment	Laboratory			Greenhouse
	Root length (cm)	Shoot length (cm)	Mean germination time (day)	Shoot length (cm)
Not pelleted	7.75 c	5.34 bc	8.19 a	4.28 bc
Pumice	12.01 ab	5.27 c	5.16 bc	3.93 c
Talcum	13.18 a	5.89 a-c	4.29 cd	4.20 bc
Bentonite	13.39 a	6.36 a	4.85 cd	4.24 bc
$CaSO_4$	12.66 a	6.41 a	4.81 cd	4.82 a
$CaCO_3$	13.33 a	5.85 a-c	4.88 cd	4.21 bc
Zeolite	10.57 b	6.12 ab	5.67 ab	4.37 b
F-test	**	**	**	**
CV. (%)	11.30	8.67	8.17	5.51

** Interactions significantly different at $P \leq 0.01$. Means within one column with different letters are significantly different ($P \leq 0.05$). Coefficient of variation, CV (%), is the percentage variation in the mean across treatments.

3.3 Accelerated ageing tests

Under laboratory conditions, seeds pelleted with $CaSO_4$, pumice, and zeolite showed statistically significantly higher germination rates compared to the other pelleting matrices (Figure 4A). Seed pelleting with talcum, bentonite, and $CaCO_3$ resulted in decreased radicle emergence compared to the other pelleting methods (Figure 4C). Under greenhouse conditions, seeds pelleted with pumice showed higher germination rates but did not differ significantly from seeds pelleted with $CaSO_4$, $CaCO_3$, and zeolite (Figure 4B). The emergence percentage showed that seeds pelleted with bentonite had a higher emergence rate than the other treatments, followed by seed pelleting with talcum and $CaSO_4$ (Figure 4D).

Seeds pelleted with $CaSO_4$ had a higher quality than those pelleted with other matrices. The pelleting of seeds with $CaSO_4$ resulted in a comparatively low friability, indicating that seed pelleting can help protect the seeds from unfavorable environmental conditions. Additionally, $CaSO_4$ contains important nutrients, such as calcium, which plays a crucial role in the synthesis of pectate and protein, and facilitates NO_3-N uptake. It also affects the activity of certain enzymes and increases protein synthesis in mitochondria (Liu *et al*., 2011). Furthermore, sulphur stimulates the activity of papainase enzymes, such as papain, bromelin, and ficin, as well as the synthesis of amino acids, including cysteine, cystine, and methionine, which are necessary for protein synthesis during seed germination (Bejandi, Sedghi, Sharifi, Namvar, & Molaei, 2009). Therefore, $CaSO_4$ is a superior pelleting matrix compared to the other treatments. Torres & Filho (2003) found that accelerated ageing resulted in a lower percentage of melon seed viability and vigour. Similarly, this experiment showed a decrease in seed quality. However, Gawande, Mohapatra, & Johnson (1980) reported that seed pelleting material prevented various unfavourable changes induced by the environment around the seed. This was demonstrated in the germination and radicle emergence percentage of $CaSO_4$ and zeolite pellets tested under laboratory conditions.

4. Conclusions

Considering the key physical properties and laboratory and greenhouse tests, the best matrix material for seeds of the hybrid cucumber variety YokKaow was $CaSO_4$ (50 g of $CaSO_4$) with the binder methylhydroxyethyl cellulose (0.3% w/w aqueous).

However, this was merely an initial experimental study. It is necessary to apply this seed pelleting formulation in different environments for a period of 9–12 months to verify the long-term quality of the seeds. Additionally, it is important to plant the tested seeds in a field to compare the effectiveness of pelleted and not pelleted seeds.

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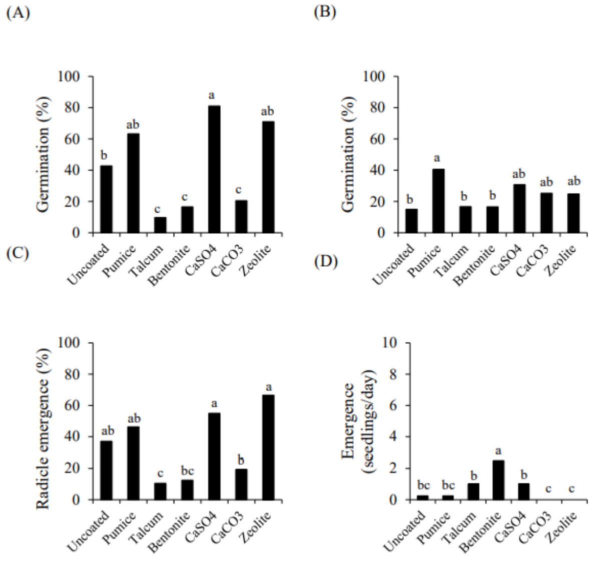

Figure 4. Germination and emergence (■) of pelleted cucumber seeds subjected to accelerated aging, in the laboratory (A and C) and in a greenhouse (B and D)

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