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The Crucial Role of Additives in the Properties of Perlite- and Gypsum-Based Superabsorbent Composites I: The Development of Composite Carrier Materials for Biological Mosquito Larva-Killing Agents

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Abstract: The increase in the risks of mosquito-transmitted serious diseases or viral infections generates strong motivations to find new and efficient solutions for controlling blood-sucking mosquitoes. There are selective protein toxins such as BTI (*Bacillus thüringiensis israelensis*) used to kill mosquito larvae, which require carrier materials that keep the active ingredient on the surface of the water where the mosquito larvae feed. Environmentally friendly and effective composite carrier materials consisting of gypsum and perlite with controlled floating and sinking times were developed. The partial closing of open pores with modified cellulose derivatives as carboxymethyl cellulose (CMC) or cricket made from corn starch and hot water were used to ensure the slow dissolution of "CMC corks" in the pores, which can control the floating and sinking properties as well. The carrier composites were combined with BTI toxins such as 4% Vectobac WP (5000 ITU (international toxic unit)) toxin, resulting in a 90–100% killing rate against different tests (*Culex pipiens*) and various naturally abundant mosquito larva species. The stability test of the BTI-containing new carrier materials shows good applicability at flooded/dried/re-flooded areas where the flooding is temporary thus the composites can be applied as preventive treatment as well.

Keywords: perlite; calcium sulfate hemihydrate; *Bacillus thüringiensis israelensis*; protein toxin; mosquito larva; floating ability; density; open and closed pores

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1. Introduction

Perlite (and pumice stone) are natural resources, amorphous glasses of volcanic origin, which have the unusual property of great expansion by heating at 850–1100 °C. The bound water content trapped in its structure vaporizes and causes 7–16-fold expansion to its original volume. The bulk densities of the expanded perlite and pumice are only 30–150 $kg \cdot m^{-3}$ [1,2]. Their composites with gypsum and cement are superabsorbents, which means they have extreme absorption capacities for water or other liquids [3,4]. The composites containing low-density powder or granular expanded perlites and pumice stones have wide industrial applications including construction, environmental, and agro-industries [5–11]. Their stability, non-toxicity [12], and high resistance against microbial attacks and organic solvents [13,14], with the use of various modifying components, make these products applicable for special purposes [15–23]. The hydraulic binders like calcium sulfate hemihydrate or Portland cement ensure an increase in the mechanical strength of the composite bodies made from perlite (or pumice) and gypsum (or Portland cement), and the structure-modifying agents such as carboxymethylcellulose, tylose, or hydroxyethylcellulose can control the ratio of the open/closed pores of the composite material [24].

The killing of mosquitoes with chemicals causes a high level of damage to other insects including bees thus great efforts were made to develop selective biological agents as protein toxins of *Bacillus thüringiensis israelensis* (BTI) and *Bacillus sphaericus* (BS) [25–28]. These toxins can selectively kill only the blood-sucking mosquito larvae, including the larvae of mosquitoes that spread serious diseases such as yellow fever, malaria, Zika, and dengue fevers. The mosquito larvae live only in specific aquatic environments, e.g., in the blank region of rivers or ponds and on the water's surface. Thus, the area where extermination of the larvae has to be performed is less by ca. one order of magnitude than the area where the adult mosquitoes are spread. These aquatic life systems generally have dense vegetation thus the spraying of active toxin solutions (available commercially (Vectobac, Vectolex)) did not lead to the expected results because the leaves of plants caught the sprayed solutions, which means the solution could not reach the surface of the water where the mosquito larvae feed. Fixing these solutions on sand, plastic, or other granules had many disadvantages [24,29–31]. The sharp edges of the sand damage the vegetation, furthermore, it sinks immediately, thus the active substance cannot release on the surface of the water. A perlite–CMC composite, which can be made in spherical form by granulation, did not cause mechanical damage to the vegetation; however, due to its high open pore content, it immediately absorbed such a large amount of water that the granules sank like sand grains [3]. Floating plastic particles are non-degradable substances that pollute the environment. The BTI proteins that are frozen into ice pellets have significant practical importance [32–34]; however, there is a series of technical limitations due to the fast melting of water in hot climatic environments where the mosquito populations are dense.

In our present work, we show the preparation, properties, and application fields of composites made from expanded perlite, calcium sulfate hemihydrate with modifying agents such as cricket made from corn starch and hot water, carboxymethylcellulose, hydroxyethylcellulose, or tylose, to prepare a low-density carrier material to carry a selective mosquito larva-killing agent (*Bacillus thüringiensis israelenses*, BTI) with a controlled floating ability in natural waters. Based on their liquid-absorbing capacities, these composites proved to be superabsorbents.

2. Materials and Methods

All chemicals (calcium sulfate hemihydrate (M = 136.14 g·mol^{-1}, Portland cement, expanded perlite, expanded pumice stone, carboxymethyl cellulose (M_w = ~90,000–150,000 g·mol^{-1}), hydroxyethyl cellulose (M_w = ~90,000–130,000 g/mol), tylose (M_w = 450,000 g·mol^{-1}), glycerol (M = 92.09 g·mol^{-1}), pentane (M = 72.15 g·mol^{-1}), and starch (M_w = 10^6–10^7 g·mol^{-1})) were supplied by Deuton-X Ltd., Érd, Hungary. Vectobac WP was supplied by Icybac Gmbh, Speyer, Germany.

2.1. Perlite-Containing Composites

A series of samples were prepared from calcium sulfate hemihydrate (155 g), perlite (15 g), 270 mL of water, and various additives such as glycerol, tylose, and carboxymethyl cellulose. The drying loss of the granulates was determined by keeping the samples in a gentle air stream for 48 h to reach constant weight (48 h). The density was measured by immersing a weighted amount of the sample into hexane, with the measurement of the volume change in the hexane corrected with the volume of the absorbed hexane (determined by the weight measurement of the hexane-immersed samples). The water-absorbing capacities were determined by immersing the sample in water for 48 h, removing the sunken granulates, and measuring their weight. The floating time of the granulates was determined visually. Compositions and several physical parameters are summarized in Table 1.

Table 1. Composition and density of floatable granules made from perlite (15 g) and calcium sulfate (155 g) in 270 mL of water with additives as modifying agents.

Additives	No Additive	1% Carboxy-Methylcellulose, Na-Salt, 30 mL	1% Tylose in Water, 30 mL, 12 mL of Glycerol
Drying loss at 25 °C in 48 h, %	57.0	55.7	56.1
Water absorbing capacity, %	145.4	133.4	90.7
Floating time, h	48	72	72
Density, $g \cdot cm^{-3}$	0.88	0.85	0.98

2.2. Preparation of Starch-Based Perlite (Pumice) and Gypsum (Cement) Composites [24]

A total of 10 kg of perlite and 20 kg of calcium sulfate hemihydrate were mixed with 0.7 kg of sodium carboxymethylcellulose (Mavibond CP-O 8000), then after granulating, the granules were dried at 80 °C for 4 h. The density of the formed granules was 0.25 $g \cdot mL^{-1}$. Its BET surface area was 47 $m^2 \cdot g^{-1}$ (Sample A). By increasing the amount of calcium sulfate hemihydrate up to 25 and 30 kg, the density of the granules increased to 0.30 and 0.35 g/mL, respectively. Instead of 30 kg calcium sulfate hemihydrate, a mixture of 20 kg of calcium sulfate hemihydrate and 10 kg of Portland cement resulted in granules with a 0.35 g/mL density. The granules formed from a mixture of 30 kg calcium sulfate hemihydrate and 10 kg expanded pumice stone with 0.7% hydroxyethylcellulose (Bermocoll E 1000) had a 0.40 g/mL density. Initially, all granules floated on the water surface; their average sinking time was close to 12 h.

First, cricket was made from 20 kg of corn starch (Hungrana Rt, Szabadegyháza, Hungary) by mixing it with 20 kg of cold water, then this suspension was poured into 240 L of boiling water. The formed cricket gel was mixed with 10 kg (100 L) of perlite powder (d = 0.1 $kg \cdot L^{-1}$) and 20 kg of gypsum (Rudagipsz Kft, Rudabánya, Hungary) under intensive stirring. Cylindrical granules were formed from the mass before solidifying, with 4 mm in length and 3 mm in diameter, then the granules were dried in air at 80 °C until constant weight was acheived. The density of the granules was 0.25 $g \cdot mL^{-1}$ (Sample B). The average sinking time was 48 h.

The same procedure was performed with 10 kg of expanded pumice (d = 0.11 $g \cdot L^{-1}$) and 15 kg of Portland cement (Duna-Dráva Cement Kft, Vác, Hungary) instead of perlite and gypsum, respectively; the density of the formed granules was 0.29 $g \cdot mL^{-1}$ in both cases. These granules floated on the surface of the water. The floating characteristics of the perlite-containing granulates were as follows: 20% was sunk in 12 h, a further 60% sunk in 24 h, and the last 20% sunk in 36 h.

2.3. BTI-Containing Granules

We have selected the cricket-containing granulates of the perlite-to-calcium sulfate hemihydrate ratio 1:2 with density d = 0.25 $g \cdot mL^{-1}$ as carrier material due to their good floating/sinking time parameters. These granules were soaked in a Vectobac WP (5000 ITU) powder suspension in 1% aqueous carboxymethyl cellulose solution and then dried. The BTI content of the dried (at 50 °C) granules and the density of the dry material were found to be 4% and 0.35 $g \cdot mL^{-1}$, respectively. The floating time varied between 12 and 48 h, and ~80% of the granules were sunk between 24 h and 48 h.

2.4. Instrumental Measurements

The PXRD patterns were acquired with the use of finely powdered samples and a Bragg–Brentano parafocusing goniometer (Manufacturer: Philips, Amsterdam, The Netherland); (CuK_α radiation, 1.5406/1.5444 Å) in the 2θ range of 4–70° (step size 0.02°, 1 s interval time) [35].

The IR spectra were collected between 4000 and 400 cm^{-1} from 16 scans and acquired with a resolution of 4 cm^{-1} (Bruker Alpha FT-IR, manufacturer: Bruker, Ettingen, Germany) in an Attenuated Total Reflectance (ATR) mode [36].

The BET (Brunauer–Emmett–Teller) surface areas were determined with the use of nitrogen adsorption data collected at −196 °C (Autosorb 1C, Quantachrome, Boynton Beach, FL, USA). The samples were evacuated at 100 °C for 24 h and then measured [37].

The morphology of the composites was studied with the use of a TESCAN VEGA COMPACT (Brno, Czech Republic) scanning electron microscope (SEM) with a 20, 2.5, and 2 kV accelerating voltage electron beam and a secondary detector at 10 mPa chamber's pressure [35].

2.5. Mosquito Larva Killing and Toxicity Test

The standard biological toxicity tests against aquatic organisms such as *Daphnia magna*, *Salmo trutta M. fario* L., and white mustard (*Sinapis Alba*) were measured in the Laboratory of Hydrobiology, Százhalombatta, Hungary, according to the OECD Principles of Good Laboratory Practices [38]. The concentration of the granules was 200 mg/L in the filtered Danube river water (pH = 7.6; dissolved oxygen 89.4 $g \cdot mL^{-1}$), with a hardness of 103 mg of CaO/L, COD ($KMnO_4$) 3.25 $g \cdot mL^{-1}$, nitrate 11.51 $g \cdot mL^{-1}$, nitrite 0.01 $g \cdot mL^{-1}$, phosphate 0.17 $g \cdot mL^{-1}$, and the temperature varied between 15 and 25 °C.

The Daphnia test was carried out according to the OECD Guidelines for the Testing of Chemicals, Daphnia SP. Acute [39]. The age of the test organisms was 1–2 days. The test period was 2 days at 20 ± 1 °C. Four experimental units and one control unit were tested.

The white mustard (*Sinapis alba*) tests were performed for 3 days using aqueous media by soaking the granules for 72 and 168 h in the Danube River water. Fifty seeds were used in every test, and four experimental and one control test were performed. The temperature was 20 ± 2 °C.

The fish test was performed according to the OECD Guidelines N. 203 (1992) [40]. The tests were carried out in 24, 48, 72, and 96 h test periods at the trout farm, Lillafüred-Garadna, Hungary. The tests were conducted in tanks containing ca. 100 fish/300 L, in 16 h light/8 h dark periods, at 15–17 °C, in the sand-filtered Danube river water, with an 80–90% air saturation level. Four experimental and one control test were conducted.

The optimal practical parameters were found for composites made according to Sample B. Sample B containing 4% BTI was used with the following parameters: 200 ITU (international toxic unit), cylindrical form, 3 mm in diameter, 4 mm in length, $d = 0.35\ g \cdot cm^{-3}$, and the average volume of the cylinders was 0.028 cm^{-3}. Consequently, the weight of one cylinder was ~0.01 g, resulting in the average covering of the one hectare water surface; ~10 cylinders$\cdot kg^{-1}\ m^{-2}$ water surface.

The efficiency of 200 ITU (4% Vectobac WP (5000 ITU)) BTI-containing granulates prepared by us (Sample B) was tested under laboratory conditions in two sets, one with *Culex pipiens* test larvae, the second with *Culiseta morsitans*, *Aedes rusticus*, *anopheles claviger*, *Aedes cataphylla*, *Aedes cantans*, and *Aedes communis* larvae collected from natural sources.

We performed small field mosquito larvae killing experiments near Zirc city, Hungary, in ponds with 20–22 m^2 surface and an average 0.4 m depth filled with natural water, with the use of isolators containing an average of 60–70 pieces/L of larvae (the largest part in L_3, the residual in L_2 and L_4 development stage). Most of the larvae found belonged *to Aedes cantants*, but larvae of other species such as *Aedes sticticus*, *Aedes cinmereus*, *Aedes capaphylla*, and *Culiseta annukate* were also present. The tests were performed with Vectobac WP (5000 ITU) containing granules, which resulted in 200 ITU active ingredient content at the applied dose and 4% BTI.

Large-field mosquito larva-killing experiments were performed near Kiskunlacháza Airport, Hungary, in natural ponds of 10 ha areas. A 3 hectare wide band in the pond systems was used for testing, which was partly (50–60%) covered with vegetation (reeds, sedges), and the depth of the water varied between 20 and 40 cm. The observed larva populations belonged to *Aedes sticticus*, *Culex modestus*, and *Culex territans* species. Further test species were added into isolators, namely the larvae of *Aedes cantants*, *Culiseta annulata*, *Culex pipins*, and *Aedes cataphylla*. The spraying was carried out with the use of an MI-2 helicopter in an 8 kg/ha dose. The spray height was 18 m, and the width of the spray was 40–50 m. There were 20 isolators in the 3 hectare area at different parts, each with 50 larvae from the naturally found and added larvae species. The minimal distance between the isolators was 100 m. After spraying, the coverage varied between 40 and 50 pieces of granules/m^2 water surface.

3. Results and Discussion

3.1. Carrier Materials from Perlite–Gypsum Additives and Perlite–Gypsum–CMC–Cricket Composites (Sample B) for Carrying Selective Mosquito Larva-Killing Biological Agent (BTI)

A series of various composites consisting of perlite (pumice stone), gypsum, cement, and various structure-modifying agents, such as cricket, glycerol, tylose, and hydroxyethyl- and carboxymethyl cellulose were prepared and tested to control their density (to be <1 g mL^{-1}) and floating ability on the water surface. Some important parameters of floating composites made without using cricket are summarized in Table 1.

The knowledge of these parameters is essential to reach the desired floating and sinking time of the granules. Substituting gypsum and perlite with cement or expanded pumice stone, respectively, increased the density with loss of the floating ability. Although the granules given in Table 1 had good floating ability, their density is relatively high, close to 1 g mL. The number of granule pieces in one kg of composite, which has *n*-times less density (it means *n*-times higher volume at the same mass), is *n*-times higher than in the reference material if the volume of each granule is the same for both composites. Therefore, in further experiments, we used cricket-containing samples, which ensures much better density values ($d < 0.35$ g·mL^{-1}). One of the composites, made from perlite and gypsum with cricket and carboxymethylcellulose as additives, gave the most effective volume/mass ratio (~4, $d = 0.25$), giving almost 4 times more granules from the same mass than the composites in Table 1, with similar floating and sinking parameters, and with the best granularity [24]. Other parameters to avoid vegetation damage, such as good conditions for spraying from an aircraft, high coverage on the water surface, fast release of protein toxin and decomposition after killing the mosquito larvae, and subsequent sinking in water were also taken into consideration. Density is one of the key factors because, at the same mass loading of the aircraft, the lower the density of the granules, increase the area that can be covered (higher volume and number of granules can be loaded).

We have not found a direct chemical reaction between perlite and calcium sulfate (Figure 1); however, the presence of carboxymethylcellulose especially together with cricket prevented the crystallization of the calcium sulfate dihydrate formed during hydration of the calcium sulfate hemihydrate (Figure 1).

The IR bands of sulfate and silicate species however appear together as a coinciding band system in the region of 1200–800 cm^{-1} in the spectra of the cricket-free perlite–gypsum sample, whereas further bands of the organic binder (cricket) appeared in addition to the previously reported band system (Figure 2). The most intensive ν_3(Si-O) bands (antisymmetric stretching mode) of the tetrahedral SiO_4 anions in the silicate components of perlite overlap with the most intensive ν_3(S-O) band (antisymmetric stretching mode) of the tetrahedral sulfate ion in calcium sulfate hydrates, resulting in a wide band system. The bands belonging to cricket ν(C=O) appeared only in the IR spectrum of Sample B around 1700 cm^{-1}.

The ratio of open/closed pores in these composites is one of the key elements to reaching the expected floating ability and sinking after releasing the protein toxin (BTI). The ratio of open/closed pores on the perlite-containing composites can primarily be adjusted with the control of the perlite-to-gypsum (or cement) and cricket ratios in the prepared composites (Figure 3). The key to the floating condition is that the density of the granules has to be below the density of the water even after filling the empty open pores with water. The closed pores contain air, which should be opened after contact with water for an adjusted time, to be filled with water to increase the density above the density of the water (Figure 3).

The solid, dashed, and dotted lines belong to three different kinds of granules with different pore sizes and content, resulting in different water-absorbing rates. The density of each dry granule increases with time (t). The penetration of water into all pores results in a density limit, which means the maximum density of the granules when all the pores are filled with water. At the time (t_1, t_2, or t_3) when the density of the granules becomes higher than the density of the water, the granules will sink. To reach this point, a part of the originally open pores in perlite or pumice should be closed by a "cork". We used moderately water-soluble conditioning agents such as carboxymethylcellulose, which can slowly dissolve in water, opening the route (channels) for the water infiltration into the originally open but temporarily closed pores. This process takes ca. 24–48 h, and the "corks" become permeable by water. When these "corked'" pores (Figure 4) are filled with water, due to the exchange of the air in these inner pores, the granules can sink (the density becomes >1 g/mL).

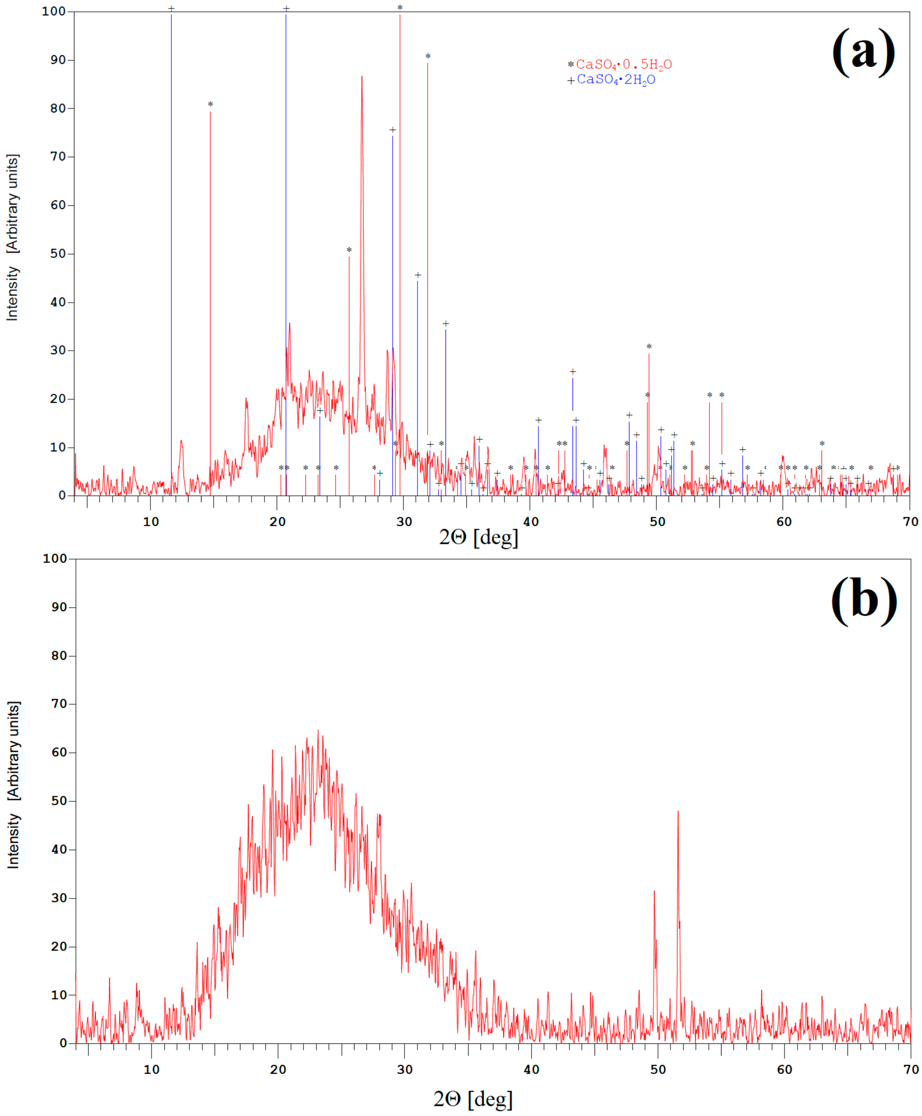

Figure 1. The PXRD of the (**a**) perlite–gypsum–carboxymethylcellulose (Sample A) and (**b**) perlite–gypsum–cricket–carboxymethylcellulose (Sample B) composites.

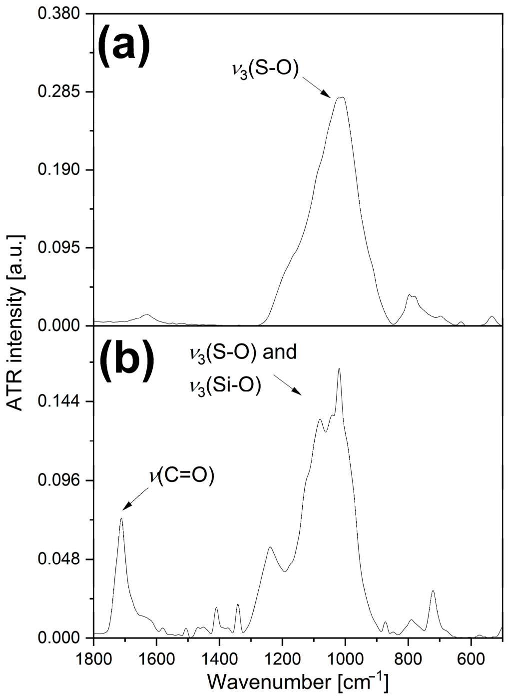

Figure 2. IR spectra of composite Sample A (**a**) and Sample B (**b**) in 1800–400 cm^{-1} range.

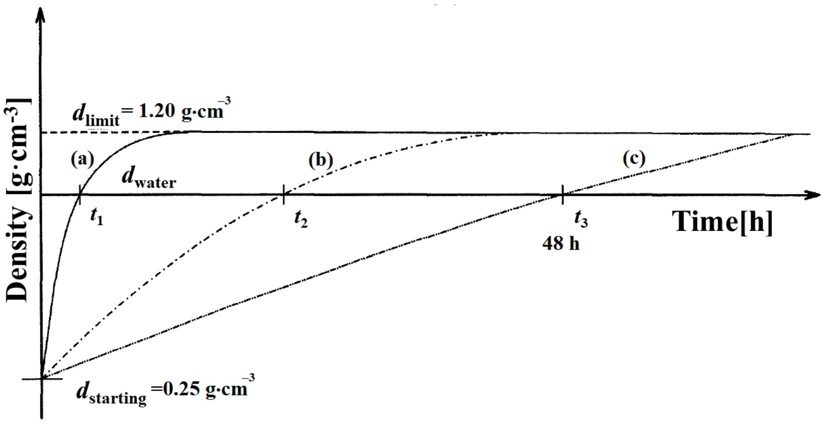

Figure 3. The variation in the density of the granules as a function of time. t_1, t_2, and t_3 mean the times when their density reaches the density of the water. The three lines (solid, dashed, and dotted) demonstrate the three kinds of granules with different pore sizes and content, resulting in different water-absorbing rates (a, b and c are curves of three kinds of granules with three different pore content).

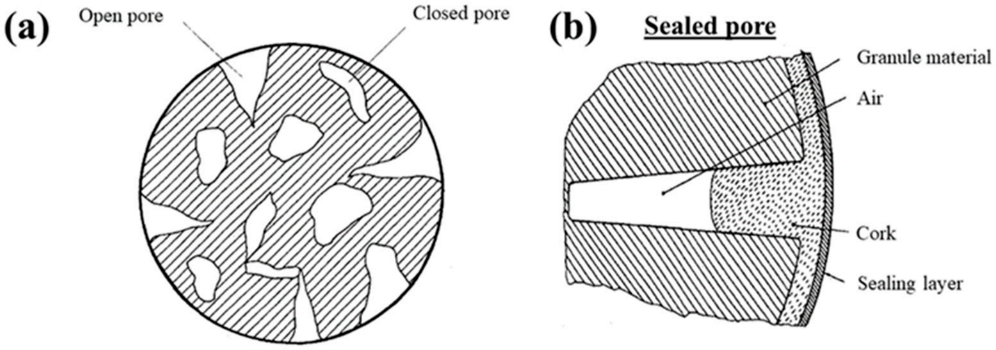

Figure 4. A schematic representation of a perlite granule with open and closed pores (**a**), and the "cork" and the outer sealing layer of the BTI–CMC–gypsum–cricket and CMC–BTI mixtures (**b**), respectively.

The optimal parameters (density, floating ability, sinking time) were found for the case of the perlite-to-gypsum ratio (by weight) 1:2, namely, the use of the same amount of starch and gypsum and the use of a five times larger amount of hot water than starch to make the cricket solution. The density and BET surface area of the cricket-containing granules were found to be 0.25 g·mL^{-1} and 27 m^2·g^{-1}, respectively, whereas the cricket-free composite had 0.35 g·mL^{-1} density and 41 m^2·g^{-1} BET surface area. The morphology of the granules' surfaces is shown in Figure 5.

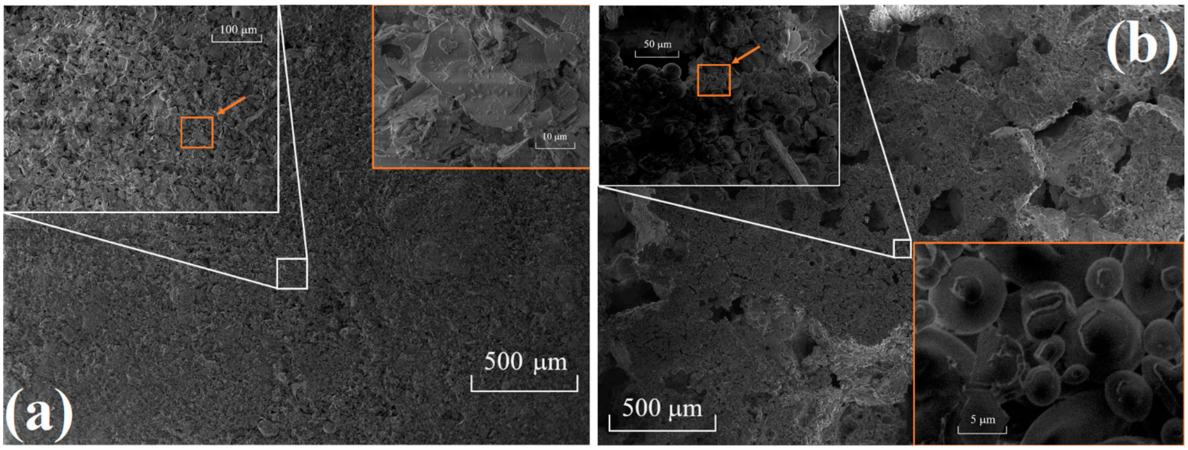

Figure 5. The scanning electron microscope (SEM) picture of (**a**) the perlite–gypsum–carboxymethylcellulose (Sample A) and (**b**) the perlite–gypsum–cricket–carboxymethylcellulose (Sample B) composite material.

The concentration of carboxymethylcellulose was ~1% in water. The protein toxin ingredient could be mixed easily with the dense honey-like consistency solutions of 1% CMC (with the use of hydroxyethylcellulose we found similar results), and a sealing layer from these CMC–BTI mixtures is also built on the external surface of the granules. After the dissolution of the external (sealing) surface layer in the water, the CMC content of the "cork" material dissolves, and water can penetrate the pores. Controlling the amount of "cork" material, together with the ratio of the open/closed pores, and the layer thickness of the external sealing material, one can adjust the floating time to be 1–2 days, which is more than enough to kill the mosquito larvae. The particles after sinking can decompose in aquatic environments in several weeks completely, the cricket content decomposes as organic material, and the silicate and gypsum content are mineral materials that do not contaminate the environment. The floating time of the granules with a cylindrical shape, 3 mm in diameter and 4 mm in length, prepared from perlite, gypsum, 1% aq. CMC, 4% BTI, and cricket were found to be 2 days on average (90% and 80% of the granules can float on water after 1 and 2 days, respectively), and practically all the granules were sunk by the end of the second day.

The size of the granules has to be uniform to ensure homogeneous distribution when air spraying. The shape and size of these granules were selected to be cylindrical, 3 mm in diameter, and 4 mm in length. The amount to be sprayed was selected to be 5, 10, and 15 kg per hectare to reduce the cost/hectare parameter with one take-off of the aircraft with a given load of the granulates. Beyond these considerations, the coverage of the water surface with the particles (pieces/square meter) should also be as high as possible. These are expected to be 50, 100, and 150 pieces·m^{-2}, at 5, 10, and 15 kg/hectare doses, respectively.

3.2. The Biological Tests of the Prepared BTI-Containing Composite (Sample B)

In the first test with *Culex Pipiens* mosquito larvae, the granules were tested in 5, 10, and 15 kg/ha doses on an open water surface and the results are summarized in Table 2. The tests were conducted with recovered granules as well, after 2, 4, and 6 days of storage in a dry environment, to test the efficiency in the flooded/drying/re-flooded field, where the flooding is temporary, and the mosquito larvae generations experience interruptions.

Table 2. Mosquito larva-killing efficiency of perlite–gypsum–cricket composite containing 4% BTI active ingredient and corked/covered with 1% carboxymethylcellulose solution (cylinders, 3 mm in diameter, 4 mm in length, d = 1.35 g·mL^{-1}, average cover is ~50, 100, and 150 pieces·m^{-2} at 5, 10, and 15 kg/ha dose, respectively) [24].

Dose, kg/ha	5	10	15
Time, days		**Efficiency, %**	
1	80	92	96
2	72	92	100
	After 2 days of dry storage		
1	96	96	98
2	96	100	96
	After 2 more days of dry storage		
1	90	88	94
2	88	99	96
	After 2 more days of dry storage		
1	88	82	92
2	88	80	84

The efficiency of the mosquito larva-killing is extremely good at 10 and 15 kg/ha doses (>90%); in 2 days, at 15 kg/ha dose, the larva-killing was complete. We repeated the tests with the granulates recovered after the first test, with two days of intermittent dry storage, which showed only a minimal decrease in efficiency in the four cycles. In the fourth cycle, the mosquito larva-killing efficiency was still above 80%.

The tests showed that the perlite–gypsum–cricket composite granules containing 4% BTI active ingredient (Vectobac WP, 5000 ITU), carboxymethylcellulose “cork”, and sealing material resulted in a high-efficiency mosquito larva-killing agent. This shows that these granules are potential candidates for mosquito larva-killing in flooded areas as well, because their activity is retained in dry periods, and can be re-activated in a new flooding period until exhausting their BTI content. Our previous experiments with clay carriers under analogous conditions showed that the presence of calcium sulfate is essential to achieve good larva-killing activity thus calcium sulfate has some role in the composite, probably due to its sulfate content [24].

A second set of tests was carried out with L_2 and L_3 stages of *Culiseta morsitans* (56%), *Aedes rusticus* (25%), and *Anopheles claviger* (12%) with *Aedes cataphylla*, *Aedes cantans*, and *Aedes communis*, 7% altogether. The tests were performed between 21 and 23 °C, and every test crucible contained 100 pieces of larvae. The living larvae were counted after 24 and 48 h. Three series of experiments were carried out with one control experiment. The results can be seen in Table 3.

The mortality was found to be excellent at 10 and 15 kg/ha doses in 24–48 h even at 50 pieces/m^2 dose. Complete mortality could be achieved at 15 kg/ha dose even in 24 h. There were no differences between the mortality of the different species. Since natural water was used for testing, numerous other spineless and amphibic organisms were detected in the water (*Chrironomus plumosus*, *Baetis rhodanit*, *Nemoura cinecerea*, *Aeshna cyanea*, *Coenagrion puella puella*, *Ischnura elegans pontica*, *Pyrrhosoma nymphula interposita*, *Notonecta gluca*, *Nepa cinerea*, *asellus aquaticus*, *Gammarus roeseli*, *Limnaea truncatula*), which were not affected by the granules used even after 48 h. Thus, the BTI-containing granulates were found to be non-toxic to these aquatic organisms. The carrier composite was also tested in standard toxicity tests [38–40], and there was no mortality in the case of the trout and Daphnia tests, and the seedlings of the white mustard were found to be 95.07% and 86.86% of the control after 72 and 168 h extraction, respectively. There was no observed swallowing by trout, despite their cricket content; the fish assigned it as a "mineral" non-food material.

Table 3. Mosquito larva-killing efficiency of perlite–gypsum–cricket composite containing 4% BTI active ingredient (200 ITU) corked/covered with 1% carboxymethylcellulose solution (cylindrical granules, 3 mm in diameter and 4 mm in length, $d = 1.35\ \mathrm{g \cdot mL^{-1}}$ with average coverage of ~50, 100, and 150 pieces per m^2 for 5, 10, and 15 kg/ha doses, respectively).

Sample	**Number of Larvae at the Beginning**	**Larva Mortality in %**	
		24 h	**48 h**
50/m^2 coverage (5 kg/ha)	100	72	99
100/m^2 coverage (10 kg/ha)	100	95	100
150/m^2 coverage (15 kg/ha dose)	100	100	100
Control	100	0	2

The natural field tests were carried out in two steps. First, a small area test was performed in ponds with 20–22 m^2 surface and 0.4 m depth, on average, and filled with natural water. The isolators contained 60–70 pieces of larvae in a liter of water on average (the largest part in L_3, the rest in the L_2 and L_4 stages). The main species was the *Aedes cantanas*, but other species such as *Aedes sticticus*, *Aedes cinereus*, *Aedes cataphylla*, and *Culiseta annulate* were also present. The average mortality was found to be 80–85% in 24 h, taking into consideration the 4–5% natural mortality, and after 48 h, the mortality became 100%.

A large-field experiment, which was practically identical to the operations planned for mosquito larva-killing under practical conditions, was performed in a pond system with vegetation (50–60%) coverage of reeds and sedges, at 20–40 cm water depth, at room temperature. The larvae tested belonged to *Aedes sticticus*, *Culex modestus*, *Culex territans*, *Aedes cantants*, *Culiseta annulata*, *Culex pipins*, and *Aedes cataphylla*. The spraying was performed from a helicopter in an 8 kg/ha dose with a 40–50 m spraying width from 18 m height. The mortality of the studied larvae was found to be 100% after 24 h.

4. Conclusions

We have shown a simple and convenient method to prepare naturally degradable gypsum–perlite–cricket–carboxymethyl cellulose composites as carrier materials for mosquito larva-killing with BTI active ingredient. These composites have enough mechanical strength to spray them from a helicopter; the cylindrical geometry of the granules decreased the air drag and ensured wide spreading ability (50 m width) with a homogeneous distribution on the surface of the water. The composites have low-density and controlled floating time. The floating time of the granules with a cylindrical shape, 3 mm in diameter and 4 mm in length, prepared from perlite, gypsum, 1% aq. CMC, 4% BTI, and cricket was found to be 2 days on average (90% and 80% of the granules can float on water after 1 and 2 days, respectively), and practically all the granules were sunk after 48 h. This floating time is enough to accomplish the almost complete killing of the mosquito larvae even in an environment covered with dense vegetation. In our biological tests, we have shown that under natural conditions, up to 100% of the *Aedes sticticus*, *Culex modestus*, *Culex territans*, *Aedes cantants*, *Culiseta annulata*, *Culex pipiens*, and *Aedes cataphylla* species could be killed in 24–48 h at a 5–15 kg/hectare dose. The stability test of the new carrier materials shows good applicability in flooded/dried/re-flooded areas where the flooding is temporary thus the composites can be applied as a preventive treatment as well.

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