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Enhancing insecticide activity of anacardic acid by intercalating it into MgAl layered double hydroxides nanoparticles

Tăng cường hiệu lực diệt sâu của anacardic acid bằng cách gắn chèn nó vào hạt nano lớp đôi hydroxides MgAl

Research article

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MgAl layered double hydroxides nanoparticles (LDHs) are known as the useful materials in agrochemsitry. LDHs can be used as a bio-insecticide carrier to enhance insecticide's activity efficiency. In our study, to improve the insecticide activity of anacardic acid, an extract from cashew nut shell liquid, we intercalated it MgAl layered double hydroxides nanoparticles. Different hybridization between anacardic acid and LDHs (37, 74, 148, and 296µg/mL) (L-As) were made and tested on the survivals of cutworms (*Spodoptera litura*). L-As or free anacardic acid was sprayed directly on the leaves mustard to feed cutworms or directly on the skin of cutworms. Our results showed that in all L-As treatments, the worm killing efficiency was higher than the free anacardic acid treatment.

Hạt nano lớp đôi hydroxides MgAl (LDHs) được biết đến như là những vật liệu hữu ích trong nông ngành hóa học nông nghiệp. LDHs có thể được dùng như là một loại chất mang cho thuốc trừ sâu sinh học để tăng cường hiệu lực diệt sâu. Trong nghiên cứu này, để tăng cường hiệu lực diệt sâu của anacardic acid, một loại hoạt chất được chiết từ dầu vỏ hạt điều, chúng tôi đã gắn chèn nó lên hạt nano lớp đôi hydroxides MgAl. Các nồng độ khác nhau của dạng lai của anacardic và LDHs (37, 74, 148 và 296µg/mL) (L-As) đã được kiểm tra tỷ lệ sống của ấu trùng sâu khoang (Spodoptera litura). Các nghiệm thức L-As và dạng anacardic acid tự do đã được phun lên lá rau cải ngọt cho ấu trùng sâu ăn hoặc phun trực tiếp lên da ấu trùng sâu. Kết quả cho thấy, tất cả các công thức có xử lý bằng L-As, hiệu lực diệt ấu trùng sâu đều cao hơn so với dạng anacardic acid ở trạng thái tự do.

Keywords: anacardic acid, MgAl LDHs, bio-insecticide, cutworm, *Spodoptera litura*

1. Introduction

LDHs, anionic clay minerals, are represented by the formular $[M^{II}_{1-x}M^{III}_{x}(OH)_2]^{x+}[A^{n-}]_{x/n}.yH_2O$, in which M^{II} and M^{III} are di- and tri-valent cations in octahedral positions and A^{n-} is an interlamella anion. Most commonly, M^{2+} is Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, ··· and M^{3+} is Fe^{3+}, Al^{3+}. The x value is the molecular ratio of $M^{2+}/(M^{2+}+M^{3+})$ and usually from 0.2 – 0.33 (Cardoso et al., 2006). For example, mostly found type of natural clay LDH is $Mg_6Al_2(OH)_{16}CO_3.4H_2O$ (Cardoso et al., 2003).

LDHs are known as nanomaterial carriers of various pesticides and herbicides to control the effect of their residues on human, the animal and the ecological environment. For instance, the intercalation of herbicide 2,4-D, MCPA, picloram into LDHs slowed down the releasing of these herbicides (Cardoso et al., 2006). Besides, the hybridization of 5-amino salicylic acid into nanoparticles Zn/Al LDHs and Mg/Al LDHs to prolonged the effect of this substance (Abadi and Rezvani, 2012).

Anacardic acid (AnAc), 6-pentadecyl salicylic acid, is the main phenolic lipid in cashew nut shell liquid (70%). It is a derivative of salicylic acid substituted with an alkyl

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chain of variable length, which may be saturated or unsaturated. AnAc can act as an antibiotic, a strong antioxidant and enzyme inhibitor prostaglandin synthase, lipoxygenase (Schultz et al., 2006; Nagbhushama, 1998; Brady, 1983; Stanley-Samuelson, 1987; Stanley-Samuelson and Loher, 1986), and tyrosinase (Stanley-Samuelson et al, 1986) in insects. Besides, the inhibition of AnAc on small insects like Colorado potato beetle was also recorded by Kubo et al. 1994.

In this study, to enhance the bio-pesticide activity of AnAc, we used nanoparticles MgAl LDH carrying AnAc to produce a nano-bio-pesticide hybrid. To test the efficiency of the hybrid LDH-AnAc, different treatments including free AnAc, LDH-AnAc hybrids and two other pesticides were experimented on cutworms (*Spodoptera litura*) and then evaluated through cutworm survival rate in each treatment.

2. Materials and methods

2.1. Materials

MgAl LDH anacardate nanoparticles (L-A hybrid) were synthesized by directly intercalating anacardic in the coprecipitation process at a ratio of Mg:Al = 2:1, pH 11-12 for 24 hours at 50^{o}C in bubbling nitrogen. The precipitations were washed and re-suspended into ditistilled water to get a L-A hybrid suspension with AnAc concentration of 12mg/mL (stock solution L-A). Different concentration of L-A hybrids was tested base on the experimental design in table 1. Besides that, abamectin 1.8EC and Lambda-cyhalothrin 2.5EC (Vietnamese Pesticide Co., Vietnam) were used as referential pesticides for L-A hybrids.

Cutworm larvae (*Spodoptera litura*) were grown from larval eggs (Faculty of Bio-Technology, Nong Lam University, Vietnam). The larvae grew up in big plastic trays containing mustard leaves (*Brassica juncea*). In all experiments, the larvae at 6 days after hatch were chosen. Experiments were performed at 28^{o}C, 12 h light and 12 h dark exposure, with 70% humidity. The treatments were imposed to larvae in two ways: direct and indirect contacts.

2.2. Method

2.2.1. Direct contact

The 6-day-old larvae were selected and experimented in plastic trays lined with blotting paper (12 larvae / tray). Larvae were treated by spraying various treatment suspensions, in which the larvae bodies and the blotting paper were thoroughly wetted. After that, treated larvae were transferred into glass bottles (3.5 cm diameter) containing leaf mustard. The glass bottles containing mustard leaves were refreshed everyday. Each treatment was repeated 3 times. The number of larval death was recorded every day.

2.2.2. Indirect contact

Leaf mustard was treated by spraying various treatment suspensions. Then, the 6-day-old cutworm larvae were fed by the treated leaves and cultured in blotting-paper-lined glass bottles. The glass bottles containing treated leaves were refreshed everyday. Each treatment was repeated 3 times. The number of larval death was recorded every day.

2.3. Statictical analysis

Data were analyzed by T-test, Minitab 16 software, with t= 95%.

Table 1. Experimental design

Treatments	Content
Control	Treated with distilled water
L-A37, L-A74, L-A148, L-A296	Infused suspension MgAl LDH, concentration 37, 74, 148, 296µg/mL
LDHs	MgAl LDHs suspension concentration = 165mg/L
Na-A148	Solution natri anacardate with AnAc concentration =148µg/mL
ABA	Solution Abamectin 1.8EC diluted at concentration 1mL/L
LC2.5	Solution Lambda-cyhalothrin 2.5 EC diluted at concentration độ 1.5mL/L

3. Results and discussion

To evaluate the insecticidal activity of L-A hybrids, we applied two methods: direct contact (spraying on cutworm skin) and indirect contact (spraying on leaf mustard leaves).

3.1. Insecticidal activity of the hybrid

After 5 days monitoring, Na-A148 (free AnAc concentration 148µg/mL) showed quite strong insecticidal acitivity. In this treatment, the survival rate of larvae was 50% (directly applied) and 39% (indirectly applied) while AnAc intercalated into MgAl LDHs with the same concentration of active ingredient, the insecticidal acitivity was 72% (directly applied) and 80% (indirectly applied). Besides, when the larvae were treated by LDHs, the larvae did not die, as its death rate was not different from that of in control treatment. The survival rate of cutworm larvae in LDHs treatment was 94% (directly applied) and 86% (indirectly applied) as compared to the referential control experiment having the survival rate of 81% after 5 days treatment. This result indicated that the worm-killing element was not LDH but AnAc (Figure 1). According to Oancea et al. 2009, nanoparticles MgAl LDH are material not only biocompatible to the vegetation but also beneficial in providing with some minerals to the vegetation growth (Choy et al., 2000). Therefore, LDHs particles gave the effect of increasing the insecticidal activity of AnAc when AnAc is intercalated in LDHs nanoparticles. Furthermore, we suggest that LDHs can be a suitable material to carry bioactive coumpounds to control their pesticide effectivity.

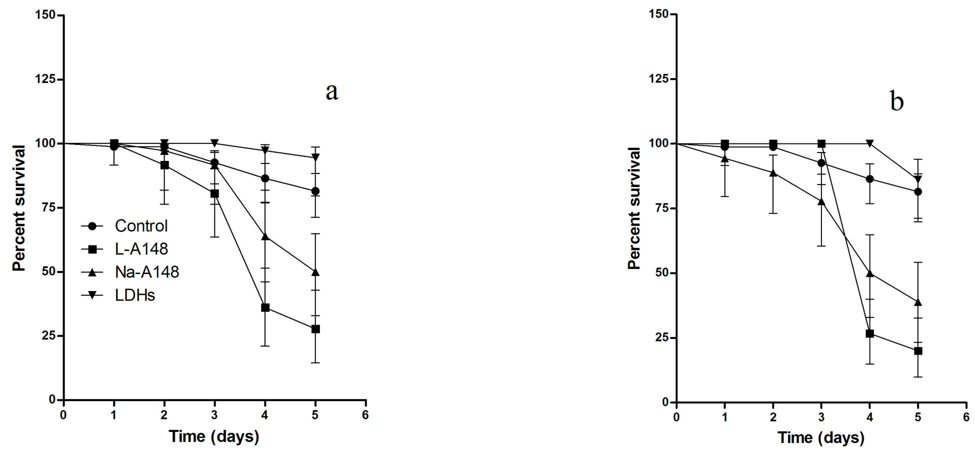

Figure 1. Survival rate of larvae after 5 days monitoring when spraying L-A 148µg/mL, Na-A 148µg/mL, Mg-Al LDHs and control by direct contact (a) and indirect contact (b)

According to Jin-Ho Choy et al. 2000, the macromolecules hardly penetrate through the cell wall; however, when they are intercalated in LDH, they become neutrally charged, and then they can easily penetrate through the cell by endocytosis mechanism and release DNA in lysosome slowly (Oance et al., 2009). Therefore, we thought that L-A hybrids have stronger and quicker effect following above-described mechanism in both methods. This has showed that, the LDH nanoparticles have the role of a carrier and distributor substance for AnAc to be more effective as shown in the obviously higher mortal rate of the larvae.

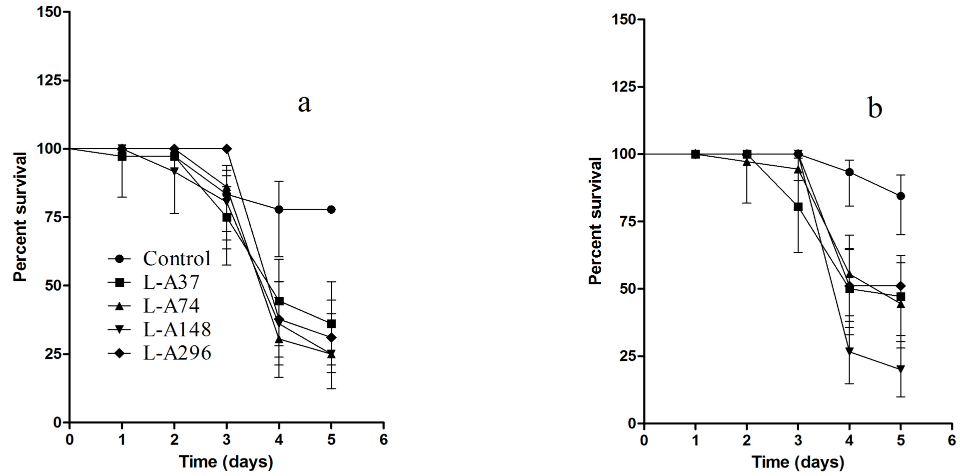

Figure 2. The survival rate of the larvae after 5 monitoring days when spraying different L-A hybrids were 37, 74, 148, and 296µg/mL by direct contact (a) and indirect contact (b)

When the larvae were treated by the different L-A hybrid concentrations (from 37 to 296µg/mL) is the result obtained in Figure 2. In contact, the survival rate of larvae in the differenent L-A hybrid concentrations decreased strongly from the 3rd to the 4th day after treated. The survival rates of L-As from 37 to 296µg/mL were 36%, 25%, 25%, and 31%, comparing with that of control was 78% after 5 days treated (Figure 2a). While the survival rates of the indirect contact were a little bit higher than that of the direct contact. The best concentration of L-A hybrids was 148µg/mL because its survival rate was 20%.

In this experiment, the larvae treated by L-As died by 2 main expressions: the most popular was the larvae ate more grew fast but, their molts were not complete, and then these cuticles ran dry and became strict rings around their bodies and finally the larvae died fast. These manifestations above may be the inhibitory tyrosinase (the molting enzyme of insect) by AnAc that Kubo and et al was found in 1994. Besides, The larvae also had the the other expression such as the molting of insect occurred regularly comparing to the control, but they ate less, grew up slowly, and died more slowly than the first expression larvae) and mostly dead for the body too weak.

3.2. Comparison of the insecticidal activity of L-A hybrid with the comercial insecticides

Active Lambda-cyhalothrin 2.5EC and Abamectin 1.8EC are two among the pesticides prescribed in the List of Pesticides of 2013 in Vietnam for controlling leaf-eating worms. Therefore, they were used as referential insecticides for the insecticidal activity of L-A148 hybrid in this experiment.

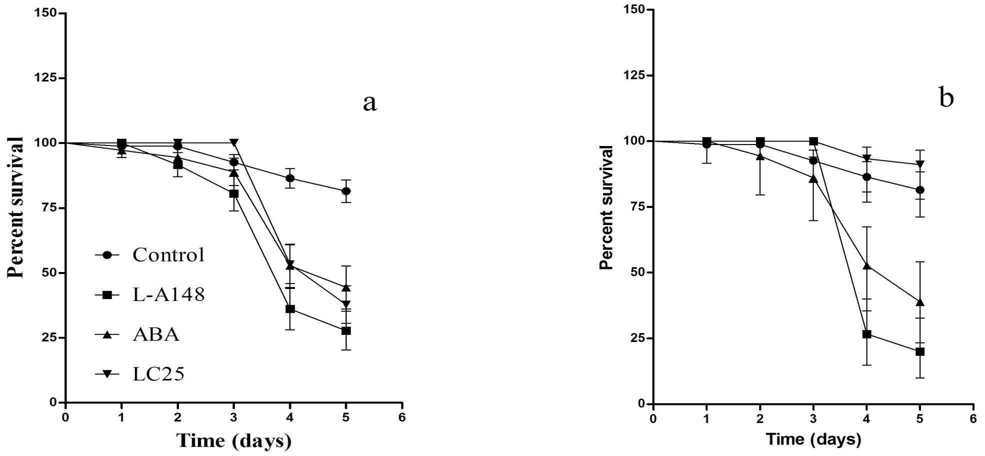

Figure 3. Survival rate of the larvae after 5 days monitoring when spraying L-A148, ABA 1.8EC dose of 1ml/L and LC2.5 dose of 1.5ml/L by direct contact (a) and indirect contact (b)

After directly spraying LC2.5 and ABA onto the larvae skin, the death rates of larvae after 5 days monitoring were 56% with ABA, 62% with LC2.5, whereas it was 72% with L-A148, as compared with 19% in the control treatment. However, when indirectly treatment through food, LC2.5 did not show insecticidal effect (death rate only 10%), while ABA and L-A148 were at 62% and 80% respectively. This result showed that, the nanoparticles LDH carrying AnAc with the dose of 148 µg/mL had stronger insecticidal activity than both LC2.5 and ABA 1.8 commercial insecticides in both applications. The effect of L-A148 was quicker, stronger than that of both comercial insecticides. This indicated that the L-A hybrid enhanced greatly the effect of the substance.

4. Conclusion

In summary, we indicated that the LDH-AnAc hybrids obtained more insecticidal activity than the free AnAc in both imposing methods, direct and indirect contacts. The highest worm killing efficiency of L-A hydrids is at 148µg/mL after 5 days' treatment. In addition, by intercalation AnAc into LDH, the L-A148 hybrid also showed higher insecticide than both ABA 1.8EC and LC 2.5 EC.

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