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Investigation of the combination of neem seed oid and rotenone extract on the inhibition of *Plutella xylostella* on *Brassica juncea*

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Abstract

The chemical pesticides being used today have adverse effects on human health and the environment. So ,they are strictly controlled, especially for export agricultural products. Since then, the search for pesticides derived from natural plants to replace chemical drugs on the market have extensively done. In this study, the combination of neem seed oil and rotenone extract on the inhibition of *Plutella xylostella* on *Brassica juncea* was investigated. The results showed that the combination of neem seed oid and rotenone extract has a significantly high insecticidal effect *in vitro* condition, the effectiveness of which is proportional to the treatment concentration, the 70% inhibitory concentration of *Plutella xylostella* was LC_{70} = 0.0130 %. In green house model , it was found that after the first treatment, the efficiency of *Plutella xylostella* control reached 38.31% and on the 7th day after the second treatment, the insecticide efficiency reached 84.81%. These results showed the high potential application of the

combination of neem seed oid and rotenone extract onvegetable cultivation to limit the use of chemical drugs, as well as the current situation of resistant pests.

Keywords: azadirachtin, combination, rotenone, Plutella xylostella L., pesticides

Introduction

The success of the green revolution in agriculture is largely attributed to the discovery and use of chemicals in pest control. The rapid increase in productivity would not have been achieved without the use of synthetic chemicals. However, the use of these chemicals also leads to serious harm to the environment and human health. Due to increasingly strict regulations, many synthetic chemicals have been restricted from usage. Therefore, the approach to using natural compounds with the ability to control pests is receiving much attention. The use of biological pesticides to replace chemical pesticides is increasingly popular. Among them, rotenone from *Derris elliptica* and azadirachin from neem tree has got much attraction.

Neem tree (*Azadirachta indica*), a species of Indian cycad is one of the plants studied quite early. The main active ingredient of this species is azadirachtin, which is very safe for humans and the environment, but toxic to many plant pests. It is one of the spearhead plants in the field of herbal pesticide research. The main effects of Neem and its active ingredient azadirachtin on pests include: internal absorption and drainage effects, growth inhibition, and stimulant effects. Asogwa et al. also demonstrated the effectiveness of neem seed extract in protecting the harmful effects of the brown planthopper *Sahlbergella singularis* in cocoa farms in Nigeria [1]. Neem seed extract was found to be effective against storage and field crop pests in Sudan and is recommended for use on small farms [2]. Another trial at the Marondera Zimbabwe Horticultural Research Center in 2011 showed that local wild plants such as Solanum, Lippia, Garlic, and Neem seeds could be formulated to help control aphids on rapeseed and is used as an organic insecticide [3]. Rotenon is also considered as one of the potential herbs to be studied as an effective insecticidal herb [4]. In 1902, Nagai first isolated a homogenate from the plant *Derris chinensis* known locally as Roten, hence

the name rotenone [5]. By 1929, Takei and Koide had determined the molecular formula of rotenone to be $C_{23}H_{22}O_6$ [6]. Rotenone is a compound that is soluble in organic solvents except CCl4, sparingly soluble in detroleum, insoluble in water. When exposed to light, air is easily decomposed from clear white to yellow, orange and crimson. Rotenone can crystallize in ethanol and some other organic solvents. Rotenone, like several other rotenoids, is highly effective against fish and many other pests, but is most effective against leafworms. The main mechanism of action of rotenone is to inhibit the multiplication of some insect cells that induce apoptosis [7]. Rotenone strongly inhibits electron transport in complex I of the mitochondrial respiratory chain. Although many research on rotenone from *Derris elliptica* and azadirachin from neem tree have done, the combination of these active component is not well-studied. In this study, the combination of neem seed oid and rotenone extract on the inhibition of *Plutella xylostella* on *Brassica juncea* was done.

Material and methods

Neem oil extraction

Neem seeds from *Azadirachta indica* were collected in Binh Thuan Province, Vietnam. They were pre-cleaned, dried and ground to size < 1.0 mm and analyzed for physical parameters (moisture, ash) according to the guidance and reference of Vietnamese Pharmacopoeia standards. The seeds were pressed to obtain essential oil (200 Mpa, 15 minutes, 40^{o}C), the neem residues after oil pressing continue to be hot-extracted with 90% ethanol to obatain the ethanol extract. The extract was then filtered and concentrated under low pressure to remove the solvent and obtain a rich of azadirachtin extract. The oil phase and the extract were mixed and homogenized to form neem oil.

Rotenone extraction

Derris elliptica were collected in Binh Duong and Bac Lieu provinces 7-8 months after planting. Raw materials are pre-treated, dried and ground to size < 1.0 mm and analyzed for physical parameters (powder, moisture, ash) according to the instructions

and reference of the standards of the company. *Derris elliptica* was then extraction with 90% ethanol at 50^{o}C for 60 min. The extract was then filtered and concentrated under low pressure to remove the solvent and obtain a rich of rotenon extract.

Qualifying the presence of azadirachtin and rotenone

For azadiractin qualification: Thin plate chromatography (TLC) was performed to determine the presence of azadirachtin in neem seed oil. The Merck SG-60 chromatographic plate was used with a mobile phase of a mixture of dichloromethane:methanol (1:1). Azadirachin (95%) (A7430 – Merck) was used as the standard. Detect the presence of azadirachtin by spraying with a mixture of vanillin reagents. The azadirachin content was quantified by HPLC chromatography.

For rotenone qualification: The rotetone content was quantified by HPLC chromatography based on Vietnamese standard (TCCS-6:2008/BVTV)

Preparing the source of *Plutella xylostella* L.

Plutella xylostella L.samples in leaf mustard (*Brassica juncea*) fields was colleted in Chau Thanh district, Tien Giang province. *Plutella xylostella* were caught, put in plastic bags containing leaf mustard. They werew then brought to the laboratory and bred on leaf mustard plants grown in potting soil, placed in specialized cages, providing food. The food were changes every 2 days to ensure adequate food supply for the worms to develop well. When the pupation was carried out, they were allowed to pair up and mate in a mesh cage supplemented with 40% honey to help the larvae develop well and lay eggs. When the eggs hatch, the larvae of the same age were separate into a plastic box and continued to breed on leaf mustard leaves until the number is sufficient, then conduct the experiment. Experiments were carried out on worms at the age of 2.

Investigation of combination of rotenone and azadirachin on *Plutella xylostella* L. in *in vitro*

The neem oil, azadirachin and the combination of neem oil and azadirachin (1:1 ratio, 0.03%) was investigated on the kill of *Plutella xylostella* L. The experiment was repeated 4 times, with 2 leaf mustard plants each time. After dropping deep into the

trees, spray the solution on the plants in the cup, each concentration sprayed 8 plants. The number of silk worms at 72 hours after spraying were count and calculated.

The effect of killing worms of the solution is calculated by the formula: M(%)= Ca – Ta/ Ca

In which: M: rate of dead worms

Ca: number of live worms in the control treatment after the experiment

Ta: number of worms living in the empirical formula

Based on the obtained results, the effective insecticidal concentration (>70%) was calculated.

Investigation of combination of rotenone and azadirachin on *Plutella xylostella* L. in green house

The leaf mustard seeds was planted on separate cassettes in the net house. Each cassette was separated by a tarpaulin so that when the silk worm is released, it could not crawl over and when spraying, it will not diffuse to the other cassette. Watering, fertilizing and taking care of the plants to grow well were done carefully and when the leaf mustard was 15 days old, the experiment was conducted.

Methods: The experiment was arranged in a completely randomized design with 6 treatments, 4 replicates, 5 m2 each. When the leaf mustard was 15 days old, 2 year old silkworms (120 heads) were released into each experimental plot the day before to stabilize. Then proceed to spray the inoculant onto the experimental plot, spray wet evenly - Monitoring criteria was observe and count the number of worms on 5 points diagonally, 5 trees each at 1, 3, 5 and 7 days after each treatment; Efficacy of herbal preparations against silkworms at 1, 3, 5 and 7 days after each treatment (%).

The potency of the drug concentrations was calculated according to the Abbott formula (1925):

Potency (H) % = [(Ca – Ta)/Ca] x 100

In there:

H: percent potency of the drug at time points.

Ca: Number of individuals living in the control treatment after treatment.

Ta: Number of individuals living in the experimental treatment after treatment.

Based on the obtained results, the effective insecticidal concentration (>70%) was calculated.

Results and Discussion

Neem seed oil extraction and qualification of azadirachin.

The neem seed material was collected, dried, and finely ground to a uniform size (<1.0 mm) (Figure 1). The physical parameters analyzed include determination of total ash reaching 4.66%; ash insoluble in HCl reached 2.91%; humidity reaches 9%. Thus, the input materials meet the requirements in accordance with the standards of granular medicinal herbs: (Total ash <6%, ash insoluble in HCl <4%, moisture from 8-10%). The azadirachin content from the neem seed oil was then qualified by HPLC. The results showed that the azadirachin content in the neem seed oil is 13.124 mg/kg. The azadirachin content was similar to previous results.

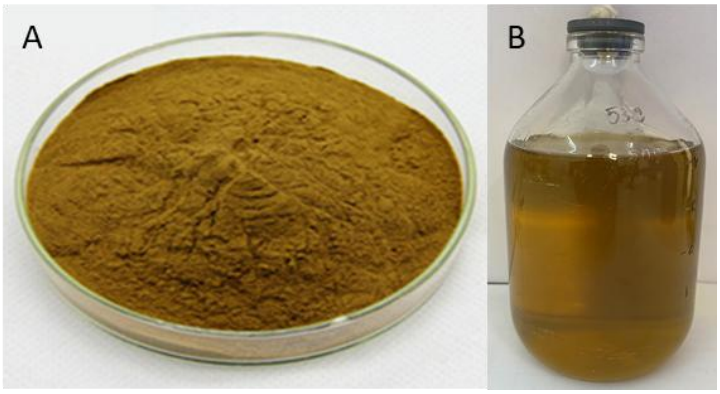

Figure 1: Neem seed sample an neem seed oil

Rotenone extraction and qualification

Derris elliptica plants were collected, dried, and finely ground to a uniform size (<1.0 mm) (Figure 2). The physical parameters were analyzed after 3 iterations, including the determination of total ash reaching 3.07%; ash insoluble in HCl reached 1.04%; humidity reaches 12%. Thus, the input materials meet the requirements in accordance with the standards of granular medicinal herbs (total ash < 6%, insoluble ash in HCl <

4%, moisture from 12-15%). The rotenone content from *Derris elliptica* was then qualified by HPLC. The results showed that the rotenone content was 3567 mg/kg.

Figure 2: *Derris elliptica* samples

Effect of combination of rotenone and azadirachin on *Plutella xylostella* L. in *in vitro*

The ability to kill silkworms of different concentrations of azadirachtin, rotenone and their combination was investigated on leaf mustard plants. The results were showed in the Figure 3. The results showed that neem seed oil and rotenone at the concentration 0.03% could kill 80.03 and 75.13% of *Plutella xylostella*, respectively. The combination of rotenone and azadirachin at the same concentration could kill 84.13% of *Plutella xylostella.* It means that the combinaton of these compound have better effect than using the single treatment.

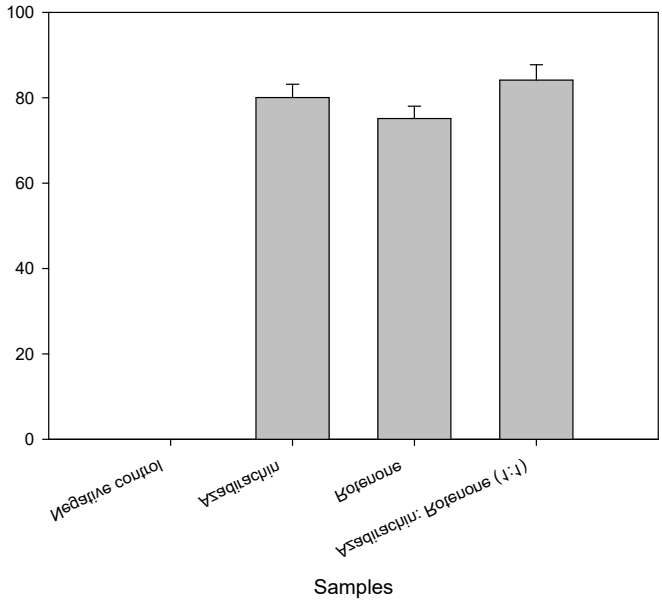

Figure 3: Effect of azadirachin, rotenone and azadirachin: rotenone (1:1) on the mortality of silk worm *Plutella xylostella in vitro.*

Effect of combination of rotenone and azadirachin on *Plutella xylostella* L. in green house

The effect of combination of rotenone and azadirachin on *Plutella xylostella* L. in green house was showed in the figure 4. In green house model , it was found that after the first treatment, the efficiency of *Plutella xylostella* control reached 38.31% and on the 7th day after the second treatment, the insecticide efficiency reached 84.81%. As showed in the figure 4, the effect of the combination azadirachin: rotenone (1:1) was 84.13% of silk worm killing. It was significant higher effect than rotenone and azadirachin alone.

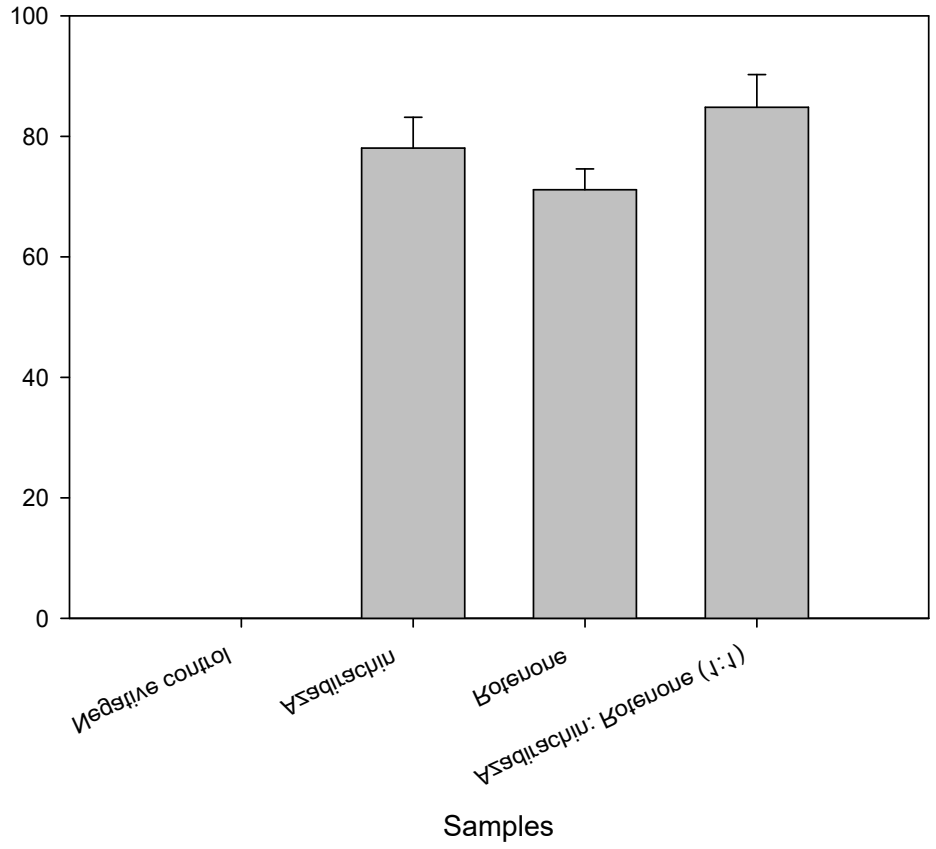

Figure 4: Effect of azadirachin, rotenone and azadirachin: rotenone (1:1) on the mortality of silk worm *Plutella xylostella in green house*

Conclusion

These results showed the high potential application of the combination of neem seed oil and rotenone extract on vegetable cultivation to limit the use of chemical drugs, as well as the current situation of resistant pests. Therefore, these combination of these plants should be used in combination with other IPM solutions in vegetable cultivation in order to limit the use of chemical drugs and limit the current situation of resistant pests.

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