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The effect of *Stemona tuberosa* root extract on preventing diamondback moths (*Plutella xylostella*) from infesting cruciferous vegetables (Brassicaceae) in the winter-spring season of 2020-2021, Thai Nguyen

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Abstract
This study examined the function and efficiency of *Stemona tuberosa* root extract as an organic pesticide against the diamondback moth on cruciferous vegetables. The 100% root extract, which was obtained via extraction by ethanol, was diluted with water in different ratios and resulted in various efficiency on eliminating diamondback larvae. Particularly, the extract diluted in ratio 1:0 was most efficient with 79.26% diamondback larvae eliminated after 4 days of application. This was closely followed by 77.04% and 76.30% from ratios 1:1 and 1:5, proving that dilution was feasible in terms of maintaining efficiency and reducing economic costs. Those results were much more prominent compared to the effect of other pesticides, which included Padan 95SP 0.01% (44.44% efficient after 4 days spraying), 1% *Sapindus saponaria* extract (24.44%), and 0.1% soap powder (22.22%). This study also showed that combined usage with those other pesticides enhanced the effect of *S. tuberosa*. The combination of *S. tuberosa* root extract (diluted with water in a ratio of 1:5) and 1% *S. saponaria* extract resulted in excellent effect with 19.61% diamondback moths eliminated after 1 day spraying and 90.18% eliminated after 7 days spraying. Although it was negligibly lower than *S. tuberosa* + Padan 95SP, this combination is completely organic and eco-friendly. In addition, *S. tuberosa* extract-based pesticides in this study were tested in cabbage fields. At the 95% confidence interval of Duncan testing, the productivity of CT_7 cabbages (34.53 tons/hectar), which were treated with *Stemona tuberosa* root extract (diluted with water in a ratio of 1:5) mixed with 1% *Sapindus saponaria* extract, and CT_6 cabbages (35.89 tons/hectar), which were treated with *Stemona tuberosa* root extract and 0.1% soap powder, was homogeneous but slightly lower than the yield of CT_8 (chemical pesticides + 0.01% Padan 95SP) with 35.75 tons/hectar.

Keywords: Diamondback, *Plutella xylostella*, *Stemona tuberosa*, Brassicaceae, *Sapindus saponaria*, Padan 95SP0, 01

1. Introduction

The diamondback moth (*Plutella xylostella*) is a member of the Plutellidae family, genus Lepidoptera. This insect is metamorphous with 4 stages of transformation (egg - larvae - pupa-adult). The life cycle of one individual only lasts 15-17 days (S. Liu *et al*., 2002) [30]. Various sources claimed that diamondback moths may have originated in Europe, Africa, or Asia (Hardy, 1938; Kfir, 1998; S. Liu *et al*., 2000; You *et al*., 2020) [23, 17, 31, 52]. Despite the debatable origin, the adult has immigrated to other regions by winds, resulting in the presence of this species worldwide. The diamond back moth has been regconized as one of the 10 most destructive insects on plants in the world, especially *Brassicaceae* spicies (Grzywacz *et al*., 2010; Tabone *et al*., 2010; Embaby & Lotfy, 2015) [16, 11, 45]. Their impacts on productivity and quality of vegetables are extremely drastic. Annually, the economic damage caused by diamondback larvae and moths could reach 4 – 5 billion USD. Since 1993, the annual cost for controlling this harmful insect has been reported at 1 billion USD worldwide and likely increasing after years (Talekar & Shelton, 1993; Zalucki *et al*., 2012) [42, 46, 53]. In Vietnam, damage from this pest is recorded year round, most significantly in the winter-spring season (Huong *et al*., 2013) [19]. Nowadays, this insect is reputable for not only huge economic damage but also their high resistance to various chemical pesticides. Recently, diamondback moths have been capable of resisting 95 types of synthetic pesticides and insecticides (Jiang *et al*., 2015) [21]. In Vietnam, this is also an alerting issue. The article of Bao Khanh & Hoang (2020) [2] reported that hundreds of Brassica hectars in Hanoi was devastated despite the fact that farmers sprayed the fields 2 times per day using various pesticides. As a result, it is clear that chemical pesticides and insecticides are no longer suitable for controlling diamondback pests. Furthermore, the use of chemical pesticides even damages the environment and creates many risks for the health of ecosystems and human beings. The study of Özkara *et al*. (2015) [39] claimed that pesticide residues have been classified as carcinogen pollutants in many countries. This is supported by other authors (Dich *et al*., 1997; Jürgens *et al*., 2015) [10, 22]. Many reports indicated that acute food poisoning could cause fatality of over 300,000 deaths worldwide (Goel & Aggarwal, 2007; Colosio *et al*., 2017) [14, 5]. Therefore, preventing the use of chemical pesticides is becoming a trend nowadays. The most suitable alternative is alledgedly bio-synthetic pesticides. In fact, the idea of using biopesticides has been studied for preventing this harmful insect. Some researchers successfully applied Deltamethrin and *Bacillus thuringiensis* in vegetable fields of Pakistan (Gong *et al*., 2010; Zamojska *et al*., 2011; Nian *et al*., 2015) [15, 54, 36]. However, diamond moths and larvae are becoming less susceptible to this combination (Shelton, 2007; Sayyed *et al*., 2008) [42, 41]. Other studies aimed to plant extracts. In Japan, Dadang & Ohsawa (2001) [7] used 4 different plant extracts from local botany and achieved an efficiency of over 81%. Begna & Damtew (2017) [3] extracted 4 different local plants in Ethiopia to successfully treat diamondback moths on cabbages. Specifically, the efficiencies on eliminating diamondback larvae were approximately fluctuating from 70% - 90%. Natural plant extracts were even used in Papua New Guinea (Oceania). The study of Iamba & Malapa (2020) [20] demonstrated that garlic extract and chilli extract did not only eliminate diamondback larvae but also prevent defoliation. The common feature of those studies is they all used local botany to make pesticides against diamondback moths. In Vietnam, there is no study utilizing this direction. This is a great opportunity for research.

With the aforementioned rationales, it is crucial to study new techniques for diamondback pest control, while also restraining their pesticidal resistance and simultaneously protecting the environment. As a result, "The effect of Stemona tuberosa root extract on preventing diamondback moths (*Plutella xylostella*) from infesting cruciferous vegetables (Brassicaceae) in the winter-spring season of 2020-2021, Thai Nguyen" was conducted to offer a solution for those issues.

2. Methodology

2.1. Materials

The primary subjects of this study included Mustard greens (*Brassica juncea*), cabbages (Brassica oleracea), Stemona tuberosa roots (tubers), and diamondback moths (pupae and adult larvae) (*Plutella xylotella*). These materials were collected in the local areas of research in Thai Nguyen Province. Other instruments required for studying were culturing cages for propagating the moth (1.2 m long, 1.0 m wide, and 0.8 m tall), experimental cages (0.7m long, 0.7 m wide, and 0.6 m tall), plastic trays for cultivating vegetables within the cages (0.6 m long, 0.5 m wide, and 0.05 m tall), and garden sprayers (vol. 1.5 l).

2.2. Research contents

There were 4 primary contents

- Determining the concentration of *Stemona tuberosa* root extract.
- Determining the content of additional ingredients supplemented to *S. tuberosa* extract.
- Determining the effects of *S. tuberosa* extract solutions on eliminating diamondback larvae
- Determining the effects of *S. tuberosa* extract solutions on cabbage productivity

2.3. Experiment design

2.3.1. *S. tuberosa* extract preparation

After collected, 1 kg of *Stemona tuberosa* roots were sliced into pieces before being ground. The raw material was then immersed in 2 liters of ethanol (45%) for 30 days to commence extraction. Afterward, the solution was filtered to obtain the extract. During the extraction time, the mixture was well agitated at least 3 times per week.

For experimenting, *S. tuberosa* extract was diluted in various ratios with water and other materials. To dilute it with water, first, the extract was stirred well. Subsequently, it was sucked into large-sized cylinders, which contained water corresponding to certain ratios of 1:0, 1:1, 1:5, 1:10, and 1:15. The diluted extract was then poured into garden sprayers.

In experiments with other pesticides, the diluted *S. tuberosa* extract was blended with pesticide Padan 95SP (0.01%), 1% *Sapindus saponaria,* or 0.1% soap powder. The mixtures were well agitated to ensure solubility.

2.3.2. Indoor experiments

1) Culturing diamonthback moths

Pupae and adult larvae of the diamondback moth were collected from local vegetable fields. When the pupae were preparing for metamorphosis, 50 – 100 individuals were selected to petri dishes, which were installed in experimental cages with trays of vegetables inside. The pupae then metamorphosed into adult moths laying eggs on the vegetables. The moths were fed with cotton balls soaked in sugared water. When the number of moths reached its peak, the process of collecting eggs begun in 3 consecutive days. The eggs were then stored in small containers. After hatching and adequately fed in 2 days, the larvae were used for experiments in 0.7 x 0.7 x 0.6 m cages (Lan Anh, 2014) [27].

2) The effect of different Stemona tuberosa root extract concentrations

Pesticides with potent bioactivity often require dilution for their applicability. Furthermore, dilution also helps preserve raw material, contributing to the economics of cultivation. This experiment was to determine the pesticidal effect of *Stemona tuberosa* root extract, which were diluted in five different ratios (1:0, 1:1, 1:5, 1:10, and 1:15), on diamondback pests. The experiment was designed in completely random patterns (Figure 1), including 6 triplicated formulas. Specifically:

- Formula 1 (CT_1) was the control formula, consisting of plants treated with only fresh water.
- Formula 2 (CT_2): plants treated with undiluted stemona tuberosa root extract
- Formula 3 (CT_3): plants treated with stemona tuberosa root extract diluted with water according to the ratio of 1:1
- Formula 4 (CT_4): plants treated with stemona tuberosa root extract diluted with water according to the ratio of 1:5
- Formula 5 (CT_5): plants treated with stemona tuberosa root extract diluted with water according to the ratio of 1:10

- Formula 6 (CT_6): plants treated with stemona tuberosa root extract diluted with water according to the ratio of 1:15.

Table 1: Experiment design of determining the effect of different *Stemona tuberosa* root extract concentrations on eliminating diamonback larvae

CT_1	CT_2	CT_3	CT_4	CT_5	CT_6
CT_6	CT_4	CT_2	CT_1	CT_3	CT_5
CT_4	CT_6	CT_1	CT_5	CT_2	CT_3

$M = \frac{Ca-Ta}{Ca}$ (%) Brassica plants (162 individuals in total with 9 per each repetition), which were at the 4th – 5th stages of growth, were installed in plastic cups sized 8 x 10 cm. The leaves from different cups were separated in terms of space. Each formula was 10 cm away from one other. Subsequently, diamondback larvae (2-day-old) were set equally on the plants (5 individuals/plant). Afterward, the treatment with *S. tuberora* extract started with one spray/day in 4 days. The number of dead and alive diamondback larvae was recorded daily. The efficiency of diluted Stemona tuberosa extract on eliminating diamonback larvae was calculated everyday (4 records in total) according to the formula of Abbott (1925) (Abbott, 1987) [1].

In this formula, M is the death rate (%); Ca is the number of larvae alive in the control formula; and Ta is the number of dead larvae in the tested formula.

3) Determining the efficiency of Stemona tuberosa root extract (diluted with water in a ratio of 1:5) combined with additives on eliminating diamondback moths

From the previous experiment on different extract concentrations, the most efficient dilution ratio, which was 1:5, was selected for this section. The experiment was to determine the most suitable additive to combine with the Stemona tuberosa root extract and result in the best efficiency on eliminating diamondback larvae.

The experiment was also designed in completely random patterns (Figure 2) including 8 formulas and 3 repetitions for each formula (8 formlulas * 3 repetition * 3 plants/repetition = 72 plants). Each plant was set with 5 2-day-old larvae, which resulted in 360 larvae in total for this experiment. Specifically, the 8 formulas consisted of:

- Formula 1 (CT_1) (Control formula 1): plants treated with fresh water
- Formula 2 (CT_2) (Control formula 2): plants treated with the 0.1% soap powder solution (10 grams of soap powder added in 10 liters of water).
- Formula 3 (CT_3) (Control formula 3): plants treated with 1.0% Sapindus saponaria extract (Extracting 10 grams of Sapindus saponaria fruits in 1 liter of water at high temperaturas)
- Formula 4 (CT_4) (Control formula 4): plants treated with the 0.01% Padan 95SP solution (1 ml of pesticide Padan 95SP added in 10 liters of water).
- Formula 5 (CT_5) (Control formula 5): plants treated with Stemona tuberosa root extract diluted with water in the ratio of 1:5
- Formula 6 (CT_6): plants treated like Formula 5 + 0.1% soap powder solution
- Formula 7 (CT_7): plants treated like Formula 5 + 1.0% Sapindus saponaria extract
- Formula 8 (CT_8): plants treated like Formula 5 + 0.01% Padan 95SP

Table 2: Experiment design of determining the effect of *Stemona tuberosa* root extract combined with different additives on eliminating diamonback larvae

CT_1	CT_2	CT_3	CT_4	CT_5	CT_6	CT_7	CT_8
CT_3	CT_8	CT_6	CT_7	CT_1	CT_4	CT_2	CT_5
CT_4	CT_7	CT_2	CT_6	CT_5	CT_8	CT_3	CT_1

Mustard greens at the 4th – 5th stages of growth (72 individuals in total) were installed in plastic cups sized 8 x 10 cm. The design of plant cups and trays corresponding to each formula resembled the previous experiment and 2-day-old diamondback larvae were put on the plants (5 individual/plant). Various pesticides including *S. tuberosa* root extract (diluted in ratio 1:5 with water), Padan 95SP, soap powder, the extract of *Sapindus saponaria*, and combinations were applied over the corresponding formula everyday in the period of 4 days. The number of dead and alive diamondback larvae was counted daily. The efficiency of the experimented solutions was recorded and calculated as the previous experiment using the Abbott's equation (Sector 2.3.1).

2.3.3. Field experiment

The field experiment was conducted during the Winter-Spring season 2020-2021 in Thai Nguyen Province. Tests were run on the Kkcross cabbage breed. In order to ensure the integrity of the experiment, all 8 formulas used in indoor experiments were selected for field evaluation, specifically:

2.3.2.1. Design

The design was completely random (Figure 3), including 8 formulas and 3 repetitions.

Table 3: Experiment design of field tests

CT_1	CT_2	CT_3	CT_4	CT_5	CT_6	CT_7	CT_8
CT_3	CT_8	CT_6	CT_7	CT_1	CT_4	CT_2	CT_5
CT_4	CT_7	CT_2	CT_6	CT_5	CT_8	CT_3	CT_1

The 8 formulas included

- Formula 1 (CT_1) (Control formula 1): plants treated with fresh water
- Formula 2 (CT_2) (Control formula 2): plants treated with the 0.1% soap powder solution (10 grams of soap powder added in 10 liters of water).
- Formula 3 (CT_3) (Control formula 3): plants treated with 1.0% Sapindus saponaria extract (Extracting 10 grams of Sapindus saponaria fruits in 1 liter of water at high temperaturas)
- Formula 4 (CT_4) (Control formula 4): plants treated with the 0.01% Padan 95SP solution (1 ml of pesticide Padan 95SP added in 10 liters of water).
- Formula 5 (CT_5) (Control formula 5): plants treated with Stemona tuberosa root extract diluted with water in the ratio of 1:5
- Formula 6 (CT_6): plants treated like Formula 5 + 0.1% soap powder solution
- Formula 7 (CT_7): plants treated like Formula 5 + 1.0% Sapindus saponaria extract
- Formula 8 (CT_8): plants treated like Formula 5 + 0.01% Padan 95SP

2.3.2.2. Implementation

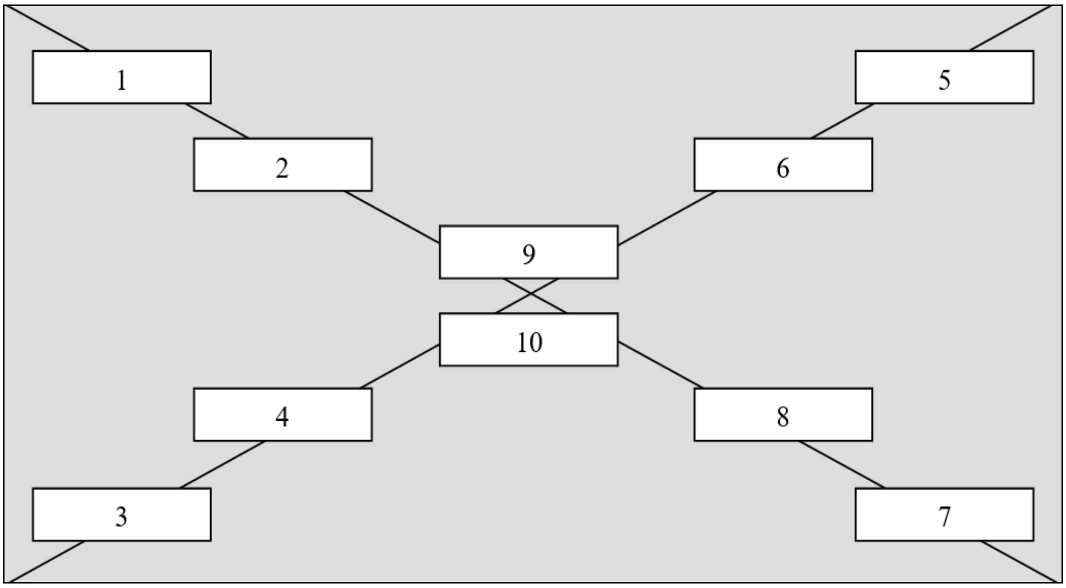

Fig 1: Outline of investigated sectors in the field.

The experiment was implemented according to the Vietnamese technical regulation on the surveillance method of plant pests (QCVN 01 – 38: 2010/BNNPTNT). Particularly, in each field or sector of a field, 10 small sectors on the 2 diagonal lines were selected for investigation (Figure 4). The surveillance process was on 2 m accross each small sector. Monitored indexes were documented in 7 days with 4 records.

2.3.2.3. Criteria for monitoring and evaluating

1) The efficiency on eliminating pests

$E = \left(1 - \frac{Ta*Cb}{Tb*Ca}\right) * 100\ (\%)$The efficiency on eliminating diamondback larvae of Stemona tuberosa extract was calculated according to the Henderson-Tilton's formula (Li *et al*., 2016) [29]:

In the formula, Ta is the number of larvae alive in the tested formula after spraying (1, 3, 5, and 7 days); Tb is the number of larvae alive in the tested formula before spraying; Ca is the number of larvae alive in the control formula after spraying (1, 3, 5, 7 days); and Cb is the number of larvae alive in the control formula before spraying.

2) The effect of Sapindus saponaria extract on the productivity of cabbages

In order to calculate the productivity of cabbage fields and formulas, the following criteria were applied after collecting data from field experiments.

$$H = \frac{\Sigma\ \text{harvested individuals}}{\Sigma\ \text{planted individuals}} * 100\ (\%)\text{-}$$

The rate of harvested plants (H):

- The average weight of cabbages (AW): Sum all the weight of cabbages in kilograms and divide by the number of plants
- Productivity per sector (P_s): Sum all the weight of cabbages in each sector to obtain productivity (kg)
- Theoretical productivity (TP):

$$TP = \frac{AW}{1000} * H * D\ (tons/hectar)$$

With D represents the density of plants (individuals/hectar)

2.3.2.4. Data analysis

Gathered data were processed by the SAS software. Graphic illustrations were synthesized using Microsoft Word 2010 and Excel 2010.

3. Results and Discussion

3.1. The effects of *Stemona tuberosa* root extract concentration on eliminating diamondback moths

Table 4: The efficiency of different Stemona tuberosa extract concentrations on eliminating diamondback larvae

Formula	Efficiency after treating (%)			
	1 day	2 days	3 days	4 days
CT_1 (Control): Treated with water	0f	0f	0e	0e
CT_2: S. Tuberosa extract (1:0)	29,63a	46,67a	65,19a	79,26a
CT_3: S. Tuberosa extract (1:1)	27,41b	41,48b	62,22b	77,04b
CT_4: S. Tuberosa extract (1:5)	22,22c	40,00c	61,48b	76,30bc
CT_5: S. Tuberosa extract (1:10)	11,85d	31,11d	50,37c	66,67c
CT_6: S. Tuberosa extract (1:15)	5,93e	14,81e	31,85d	38,52d
$LSD_{.05}$ =				

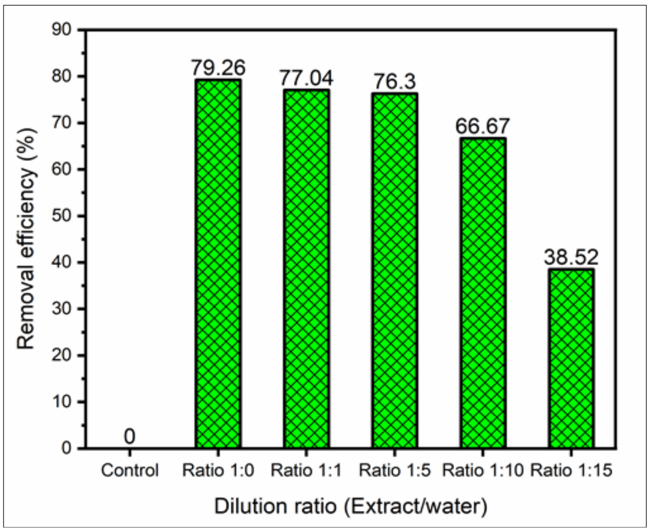

Fig 2: Graphic illustration on the efficiency of different S. tuberosa extract concentrations after 4 days

Table 1 and Figure 1 show that at different concentrations, Stemona tuberosa extract exhibited a significant effect on eliminating harmful diamondback larvae compared to the control formula, which only used water to treat the plants. More specifically, the rate of elimination and the efficiency of CT2 (1:0 ratio) were highest with 29.63% larvae eliminated after 1 day and 79.26% eliminated after 4 days. CT2 was followed by CT3, which was the extract diluted in a ratio of 1:1 with water. This formula resulted in 27.41% larvae dead after 1 day and 77.04% after 4 days of treatment. For formula CT4 (1:5), at the 95% confidence interval, the Duncan testing showed that although the efficiency was lower than CT3 and higher than CT5 (1:10) in the first 2 days, there was no significant difference in the data gathered after 4 days of treatment between CT4 – CT3 (value b) and CT4 – CT5 (value c). Finally, CT6 with the extract diluted in a ratio of 1:15 was the least efficient treatment with only 5.93% larvae eliminated after 1 days and 38.51% after 4 days. The maximum efficiency of *Stemona tuberosa* root extract on eliminating diamondback larvae in this study resembles many other botanical extracts in other studies. Specifically, it is close to the range of efficiency of tumeric and garlic extract (70 – 80%), which were tested in the study of Begna & Damtew (2017) [3]. On the other hand, *S. Tuberosa* extract exhibited a better result compared to local japanese herbs, which only reached a maximum efficiency of 71% in the study of Dadang & Ohsawa (2001) [7].

As a result, the less diluted the extract was, the more efficient it would be on eliminating diamondback larvae. Nevertheless, as the efficiency of CT3 was no much lower (only by 2.22%) than the pure extract (CT2) and there was no statistical difference between CT3 and CT4, the ratio of 1:5 was concluded to be the most suitable concentration of Stemona tuberosa extract for subsequent experiments. Furthermore, this offered more economical values on raw materials. In reality, virtually all research involving natural pesticides agreed that dilution is essential for realistic application of plant extracts (Cruz-Estrada *et al*., 2013; Nxumalo *et al*., 2021) [6, 38]. Prominently, the study of Tembo *et al*. (2018) [48] clearly demonstrated both practical and economic benefits from diluted extracts from 6 different plants. For the subject of diamondback moths, various indigenous plants from Africa and Asia were extracted for pesticidal properties. Many successful studies regarding this matter such as those of Dadang & Ohsawa (2001) [7] and Mazhawidza & Mvumi (2017) [33] indicated that ratios from 1:4 – 1:20 (5% - 20%) were the appropriate range for diluting pesticidal plant extracts regardless of plant origins.

3.2. The effects of additives on eliminating diamondback moths

Table 5: The efficiency of S. tuberosa extract (diluted in a ratio of 1:5 with water) combined with additives

Formula	Efficiency after treating (%)			
	1 day	2 days	3 days	4 days
CT_1 (Control 1): Water	$0{,}00^f$	$0{,}00^f$	$0{,}00^f$	$0{,}00^f$
CT_2 (Control 2): 0,1% soap powder	$2{,}22^e$	$8{,}89^e$	$13{,}33^e$	$22{,}22^e$
CT_3 (Control 3): 1,0% *S. saponaria* extract	$2{,}22^e$	$6{,}67^{ef}$	$13{,}33^e$	$24{,}44^e$
CT_4 (Control 4): 0,01% Padan 95SP	$8{,}89^d$	$15{,}56^d$	$31{,}11^d$	$44{,}44^d$
CT_5 (Control 5): *S. tuberosa* extract 1:5	$22{,}22^c$	$40{,}00^c$	$60{,}00^c$	$75{,}56^c$
CT_6: CT_5 + 0,1% soap powder	$26{,}67^{bc}$	$51{,}11^b$	$75{,}56^b$	$88{,}89^{bc}$
CT_7: CT_5 + 1,0% *S. saponaria* extract	$28{,}89^b$	$51{,}11^b$	$77{,}78^b$	$91{,}11^b$
CT_8: CT_5 + 0,01% Padan 95SP	$33{,}33^a$	$66{,}67^a$	$84{,}44^a$	$97{,}78^a$
$LSD_{.05}$ =				

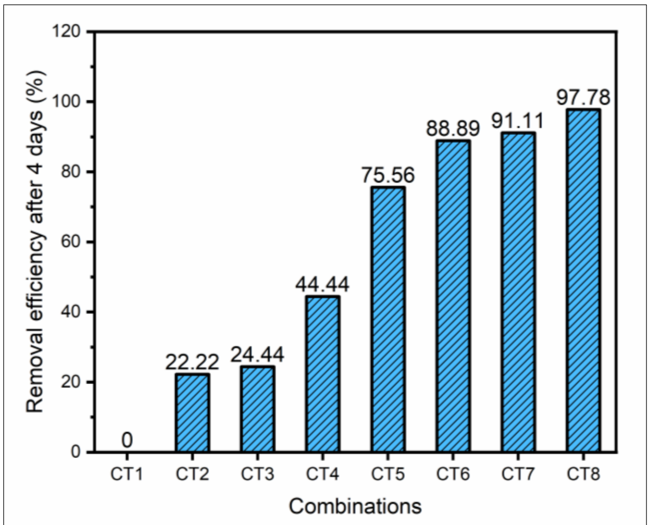

Fig 3: Graphic illustration on the efficiency of S. tuberosa extract combined with different additives after 4 days

Table 2 and Figure 2 indicate that employed additives including 0.1% soap powder, 1% *Sapindus saponaria* extract, and pesticide Padan 95SP provided certain efficiency on eliminating diamondback larvae compared to no effect from Control formula 1 (water). In general, the effects of those additives increased through each day spraying. Pesticide Padan 95SP resulted in the most effective additive as it eliminated 8.89% larvae after the first day and 44.44% after the 4th day. This is lower significantly lower than the performance of *S. tuberosa* extract (CT5). The primary constituent of Padan 95SP is cartap. Many reports from the beginning of the 21st century have indicated the rapid development of cartap resistance particularly in diamondback moths (Vastrad, 2002; Ninsin, 2015; Sunitha *et al*., 2020) [49, 37, 44]. Nevertheless, the efficiency of Padan 95SP was almost twice as high as the efficiencies of 0.1% soap powder and 1% *S. Saponaria* extract (22.22% and 24.44%, respectively), which were statistically alike in Duncan testing in the 95% confidence interval. *Sapindus saponaria* fruit extract has been employed for eliminating diamondback moth on kale in the study of Fitossanidade *et al*. (2005) [13]. However, it required a lavish amount of 10% extract to achieve desirable mortality. That explained the relatively low efficiency of *S. saponaria* extract in this study.

When combining those additives with *Stemona tuberosa* root extract (diluted in the ratio of 1:5 with water) substantailly elevated their ability to eliminate diamondback larvae. CT_6 (soap powder + *S. Tuberosa* extract) had the efficiency of 26.67% after the first day and 88.89% after the 4th day, which was approximately 4 times higher than CT_2. CT_7 (*S. Tuberosa*

+ *S. Saponaria*) eliminated 28.89% larvae after 1 day and 91.11% after 4 days, statistically resembling CT_6 and 3.7 times higher than the efficiency of only *S. Saponaria* extract. CT_8 (*S. tuberosa* + 0.01% Padan 95SP) even resulted in 97.78% larvae dead after 4 days, which was the highest efficiency recorded in this experiment, 2.2 times as high as the sole Padan 95SP treatment of CT_4. Compared to the Control formula CT_5 (sole *S. Tuberosa* extract), which resulted in 22.22% after 1 day and 75.56% after 4 days, those numbers of CT_6, CT_7, and CT_8 increased by 4.45 – 11.11% after the first day and by 13.33 – 22.22% after 4 days. All the aforementioned results were tested in the 95% confidence interval. Consequently, it is clear that the combined use is more effective than using ingredients separately. Moreover, the efficiency can reach desirable numbers in a relatively short time with more than 90% diamondback larvae eliminated after only 4 days. The trend of integrating plant extracts and synthetic pesticides remains in development with few studies in recent years (Fekri *et al*., 2016; Lengai *et al*., 2020; Khorrami & Soleymanzade, 2021) [12, 28, 24]. Nevertheless, the prospect it offers is undeniably promising. Particularly, the study of Souto *et al*. (2021) [43] asserted that with the combined force of plant extract and synthetic constituents, the mechanisms of inhibition vary in different channels and modules.

3.3. The effect of *Stemona tuberosa* root extract on eliminating diamondback moths in field tests

To further verify the ability of *Stemona tuberosa* root extract combined with additives, all the 8 formulas tested in the previous indoor experiment were employed for evaluation in realistic vegetable fields. More specifically, the 8 formulas included 1 Control formula CT1 that used water, 3 Control formulas CT_2, CT_3, and CT_4 that used the 3 additional ingredients separately (0.1%% soap powder, 1% *Sapindus saponaria*, and 0.01% Padan 95SP), 1 Control formula CT_5 that used only *S. tuberosa* root extract diluted in the ratio of 1:5 with water, and 3 tested formulas CT_6, CT_7, and CT_8 that used the combinations of *S. tuberosa* extract with the 3 additional ingredients. The results are shown in Table 3 and Figure 3 as follows:

Table 6: The efficiency of the 8 formulas in the field

Formula	Efficiency after treating (%)			
	1 day	3 days	5 days	7 days
CT_1 (Control 1): Water	$0{,}00^h$	$0{,}00^g$	$0{,}00^g$	$0{,}00^g$
CT_2 (Control 2): 0,1% soap powder	$3{,}92^g$	$18{,}07^f$	$24{,}58^f$	$28{,}22^f$
CT_3 (Control 3): 1,0% *S. saponaria* extract	$3{,}92^f$	$19{,}28^e$	$25{,}42^f$	$28{,}83^f$
CT_4 (Control 4): 0,01% Padan 95SP	$7{,}84^e$	$19{,}28^e$	$28{,}81^e$	$30{,}67^e$
CT_5 (Control 5): *S. tuberosa* extract 1:5	$9{,}80^d$	$26{,}51^d$	$55{,}93^d$	$59{,}51^d$
CT_6: CT_5 + 0,1% soap powder	$15{,}69^c$	$54{,}22^c$	$75{,}42^c$	$86{,}50^c$
CT_7: CT_5 + 1,0% *S. saponaria* extract	$19{,}61^b$	$61{,}45^b$	$83{,}05^b$	$90{,}18^b$
CT_8: CT_5 + 0,01% Padan 95SP	$23{,}53^a$	$65{,}06^a$	$88{,}14^a$	$93{,}25^a$
$LSD_{.05}$				

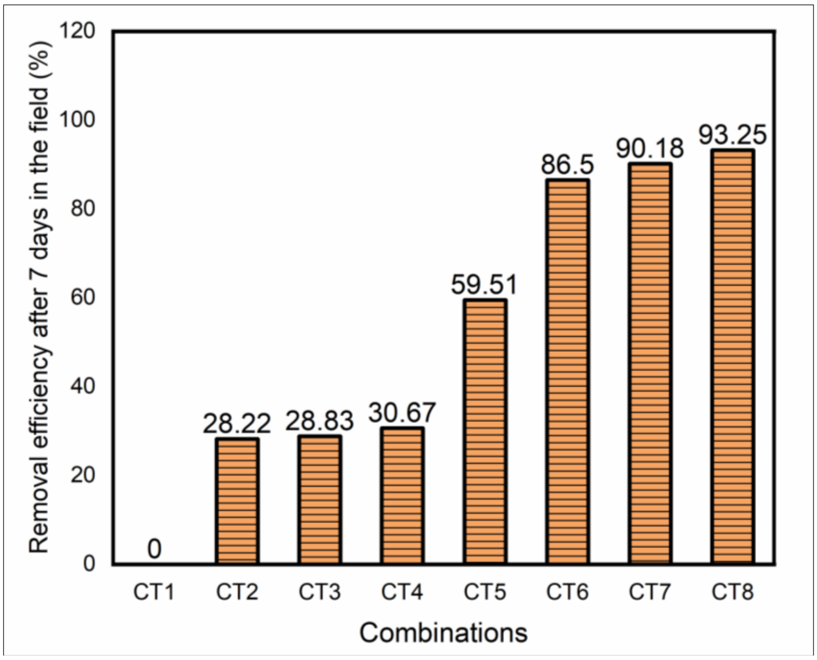

Fig 4: Graphic illustration of the 8 formulas' efficiency after 7 days in the field

Similar to the indoor experiment, the formulas that used combined ingredients all resulted in superior effects compared to control formulas. The combination of *Stemona tuberosa* root extract and 0.01% Padan 95SP still offered the best performance, followed by *S. tuberosa* extract + 1% *S. saponaria* extract and *S. tuberosa* extract + 0.1% soap powder (CT_7 and CT_6). For the separately used additives, Padan 95SP was most efficient, followed by *S. saponaria* extract and soap powder. As a result, the difference among all the tested ingredients had been further asserted.

Nevertheless, the rate of effect and efficiency were virtually lower than those recorded indoor. After the first day, the maximum efficiency recorded from CT_8 was 23.52% larvae dead, decreasing by approximately 1.42 times compared to its performance indoor (33.33%). Other formulas also performed less efficiently than the indoor experiment by 1 – 9% after 1 day. Exceptionally, day 1 in the field recorded better efficiencies from CT_2 and CT_3 (both 3.92%) compared to themselves indoor (2.22%). This tendency continously occurred for the rest of the field experiments. When the maximum efficiencies obtained after 7 days of CT_2 and CT_3 were slighly higher than those recorded after 4 days indoor, other formulas observed a decline. Despite remaining as the most efficient formula, CT_8 only eliminated 93.25% larvae after 7 days compared to 97.78% after only 4 days indoor. The performances CT_6 and CT_7, which were 88.89% and 91.11% respectively only reduced slightly compared to 86.50% and 90.18% indoor. On the other hand, the efficiency of Control formula CT_5 decreased drastically from 75.56% after 4 days indoor to 59.51% after 7 days outdoor. In addition, the performance of 0.01% pesticide Padan 95SP in the field also recorded a decline by 13.77% compared to in the laboratory.

In summary, the effect on eliminating harmful diamondback larvae of *Stemona tuberosa* root extract reached the peak when combined with Padan 95SP. However, the employment of this formula in realistic use is not recommended as Padan 95SP is a chemical pesticide unfriendly to both agricultural cultivation and the environment. In 2012, Wang *et al.* [51] examined 10 different synthetic pesticides including cartap-based ones and showed that the longevity of vegetables was substantially shortened. As a result, the combination of *S. tuberosa* and *S. saponaria* is recommended in future research and usage. Many studies focusing on pesticides indicated that Cartap, the primary component of Padan pesticides, produced residue that was virtually non-degradable (Dai *et al*., 2019, 2020; Hoang & Holze, 2021) [9, 18]. Furthermore, it has been detected in many agricultural sites worldwide. Agricultural products including vegetables, tea, and fishes are all capable of containing Cartap or its toxic metabolites (W. Liu *et al*., 2012; Vivek *et al*., 2016; Dai *et al*., 2019) [32, 50, 8]. This is a nereistoxin that promotes neuromuscular toxicity, which leads to contraction and respiratory failure (Boorugu & Chrispal, 2012) [4]. Although Cartap poisoning cases are relatively rare, the studies of Kumar *et al*. (2011) [55] and Boorugu & Chrispal (2012) [4] identified several cases with aggregated Cartap in the body, causing nausea and dyspnea. More dramatically, Kurisaki *et al*. (2010) [26] even indicated possible fatality from Cartap poisoning as the nereistoxin blocked ion exchange within the body and resulted in organ failure.

On the other hand, many studies have utilized crude plant extract for pest control (Rahman *et al*., 2016; Tembo *et al*., 2018; Tavares *et al*., 2021) [40, 47, 48]. They showed that these bio-pesticides are easily degraded and friendly to the environment. This is why in this study, CT_7 with *S. tuberosa* extract combined with *S. saponaria* extract was recommended for future use. Furthermore, the shortage in efficiency of CT_7 compared to CT_8 was relatively inconsiderable.

3.4. The effect of *Stemona tuberosa* root extract on the productivity of cabbages in the winter-spring season in Thai Nguyen 2021

Besides evaluating the ability of 8 formulas to eliminate diamondback larvae, this study also investigated their effect on the productivity of cabbages. The results are displayed in Table 4 and Figure 4 as follows:

Table 7: The effect of Stemona tuberosa extract solutions on the productivity of cabbages

Formula	*Theoretical productivity* (*tons/hectar*)	*Realistic productivity* (*tons/hectar*)
CT_1 (Control 1): Water	20,25^g	13,50^f
CT_2 (Control 2): 0,1% soap powder	22,53^f	18,02^e
CT_3 (Control 3): 1,0% *S. saponaria* extract	27,04^e	18,65^e
CT_4 (Control 4): 0,01% Padan 95SP	31,91^d	24,55^d
CT_5 (Control 5): *S. tuberosa* extract 1:5	33,05^c	28,17^c
CT_6: CT_5 + 0,1% soap powder	44,82^b	34,53^b
CT_7: CT_5 + 1,0% *S. saponaria* extract	44,86^b	34,89^b
CT_8: CT_5 + 0,01% Padan 95SP	46,47^a	35,75^a
$LSD_{.05}$		

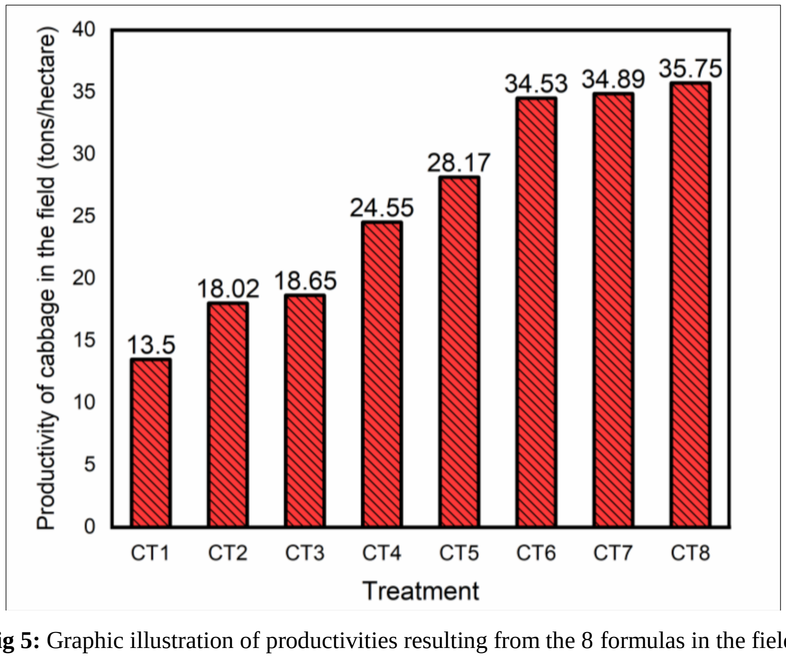

Fig 5: Graphic illustration of productivities resulting from the 8 formulas in the field.

As water had no effect on pest control, Control formula CT_1 produced the lowest productivity of only 13.5 tons of cabbages per hectar. Conseuquently, other control formulas excelled at productivity. CT_2 resulted in 18.02 and 18.65 tons/hectar, which were statistically equal to CT_3 with 18.65 tons/hectar. CT_4 using Padan 95SP produced 24.55 tons/hectar and *Stemona tuberosa* extract (1:5) produced 28.17 tons/hectar. These numbers indicate that *S. tuberosa* extract is completely capable of promoting cabbage productivity in agriculture compared to conventional chemical pesticides such as Padan 95SP. This result also resembles the study of Lan Anh (2014) [27]

All the 3 combined formulas produce higher productivities than those of control formulas. CT_6 and CT_7, which combine *S. tuberosa* extract with soap powder and *S. saponaria* extract gave statistically equal results of 34.53 and 34.89 tons/hectar, respectively. CT_8 with *S. tuberosa* extract and Padan 95SP still outperformed other formulas with the highest productivity of 35.75 tons/hectar. Nevertheless, the number was closely fit to those of CT_6 and CT_7. These results were obtained and analyzed in the 95% confidence interval. Therefore, the application of *S. tuberosa* root extract combining with other ingredients is feasible, promoting productivity of cabbages in particular. For other types of vegetables, this potent extract requires more studies for clarification.

The superiority of plants treated with *S. tuberosa* extract-based pesticides compared to untreated plants has been anticipated as there have been many studies recorded similar effects only with different plants employed in different locations. The study of Mishra & Singh (2014) [35] reached the same conclusion while testing leaf extracts from 4 different indigenous plants at cabbage fields in Northern India. However, the maximum effect achieved from neem (*Azadirachta indica*) was 56.08%, significantly lower than the finding with *S. tuberosa* extract in this study and consequently resulting in modest yield of cabbage in the field. In Oceania, Iamba & Malapa (2020) [20] studied the pesticidal effect of extracts from chili, garlic, and seaweed on diamondback larvae at cabbage field and observed reduced defoliation, which resembles the observation of this study. In addition, there are many other remarkable studies that have further proved the role of plant extracts in reducing diamondback larvae while increasing the yield of cabbage and brassica vegetables in various regions from Asia to Africa (Begna & Damtew, 2017; Mazhawidza & Mvumi, 2017; Lengai *et al*., 2020) [28, 34]

4. Conclusion

This study has successfully verified and tested the ability of *Stemona tuberosa* root extract as an effective pesticide for the diamondback moth. Particularly, all the solutions containing the extract were capable of eliminating the harmful larvae on brassica plants. In general, from both the indoor experiment and the field experiment, the efficiency increased through time. After 4 days treating, the efficiency of pure *S. tuberosa* extract (1:0 dilution ratio) was highest, reaching 79.26%. The dilution ratios of 1:1 and 1:5 also resulted in significant efficiencies with 76.30% and 77.04%, respectively, followed by 66.67% larvae eliminated by 1:10 S. tuberosa extract. Finally, the 1:15 ratio resulted in the least efficiency with only 38.52% larvae dead.

Other additives, including 0.1% soap powder, 1% *Sapindus saponaria* extract, and 0.01% pesticide Padan 95SP were also tested as means for comparisons and combinations. Padan 95SP, as a chemical pesticide, resulted in the highest proportion of diamondback larvae eliminated with 44.44% after 4 days spraying. The *S. saponaria* extract resulted in an efficiency of 24.44%, followed by the least effective additive, soap powder, with only 22.22% larvae eliminated after 4 days. When combined with the 1:5 *Stemona tuberosa* root extract, the new pesticidal solutions were effective in both the laboratory scale and the field scale. The *S. tuberosa* extract combined with 0.01% Padan 95SP resulted in the highest efficiency of eliminating diamondback larvae with 97.78% larvae eliminated after 4 days in the indoor experiment and 93.25% after 7 days in the field experiment. The combination of *S. tuberosa* extract and 1% *Sapindus saponaria* extract (CT_7) also produce a fast and strong effect when it eliminated 91.11% larvae after 4 days treating indoor plants and 90.18% after 7 days treating plants in the field. Soap powder was least effective when combined with *S. tuberosa* root extract as the efficiency was only 86.50% after 7 days in the field, though it was relatively as efficient as CT_7 in the laboratory scale, which was indicated by Duncan testing.

The use of the aforementioned pesticides also influenced the productivity of brassicaea vegetables, particularly cabbages. *Stemona tuberosa* root extract (diluted with water in a ratio of 1:5) combined with 1% *Sapindus saponaria* extract (CT_7) and 0.1% soap powder (CT_8) resulted in productivities of 34.89 tons/hectar and 34.53 tons/hectar, respectively, which were not statistically different in Duncan testing (value b). They were lower than the productivity of plants treated with *S. tuberosa* extract and 0.01% Padan 95SP, which produced 35.75 tons/hectar. In general, these numbers provide adequate confidence for applying *Stemona tuberosa* root extract in pest control and future organic agriculture. It is clear that the method of using Stemona tuberosa extract combined with 1.0% of Sapindus saponaria extract (CT_7) offered the rate of effect and efficiency equivalent to many popular chemical pesticides on the Vietnamese market. Nevertheless, it is much more beneficial when it provides no harmful effects and residues to the environment. This is also the key advantage of CT_7 over CT_8, which consisted of the extract and 0.01% Padan 95SP as Padan 95SP is a toxic pesticide, though it offered the best efficiency compared to other formulas.

Nevertheless, limitation remains with the pesticidal mechanisms of *Stemona tuberosa* extract unclear. This is a good opportunity for future research and development in order to popularize the plant extract in pest control.

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