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Development of biopesticide from bottle gourd (*Lagenaria siceraria*) leaves and black plum (*Syzygium cumini*) leaves for eco-friendly pest control in cabbage and potato fields

キャベツ畑やジャガイモ畑における環境に優しい害虫防除のためのユウガオ（***Lagenaria siceraria***）葉とクロウメ（***Syzygium cumini***）葉 由来の生物農薬の開発

Hokkaido University　Graduate School of Agriculture

Frontiers in Production Sciences　Doctor Course

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Abstract

Synthetic pesticides can have negative effects on human health and the environment. Ecofriendly biopesticides offer a potentially safer alternative to synthetic pesticides by targeting specific pests, biodegradable and leading to less pollution. Plant extracts tend to have broad-spectrum activity, are relatively specific in their mode of action, and are easy to process and use at the farm-level. Therefore, the present study was carried out to determine the efficacy of a bottle gourd leaf and black plum leaf extract in controlling cabbage looper and 28-spotted ladybird on field-grown cabbage and potato respectively, to determine the effects of the extract on the growth and yield of cabbage and potato, and to analyze the biopesticide compounds of the bottle gourd leaf and black plum leaf.

Cabbage (*Brassica oleracea* L.) crops are frequently attacked by cabbage looper larvae (*Trichoplusia ni*), which severely reduce cabbage production. This experiment was conducted in the late spring crop season from April 15, 2024, to August 20, 2024, at the Field Science Center for the Northern Biosphere, Hokkaido University, Japan. In this study, the efficacy of an ethanolic extract of bottle gourd (*Lagenaria siceraria* (Molina) Standl.) leaves was compared with those of the chemical insecticide permethrin 3.2 EC and aqueous dimethyl sulfoxide (DMSO; the control). The experiment was carried out using a randomized complete block design in which each treatment comprised three replications consisting of 24 plants in total. Compared with the control, the bottle gourd leaf extract had high biopesticide activity against cabbage looper, increasing the cabbage yield by 15.98%. The yield was almost equal to that achieved with Permethrin 3.2 EC. Compared with the control, the application of the extract significantly decreased looper larval infestation in field-grown cabbage by 41.18%, 39.71%, 52.08%, and 37.96% at the fifth, sixth, seventh, and eighth weeks, respectively. Five possible biopesticide compounds, namely, apigenin-7-*O*-glucoside, indole-3-butyric acid, strychnine, phytol and hexadecanoic acid, were identified in the ethanolic extracts of bottle gourd leaves by liquid chromatography–mass spectrometry analysis. Bottle gourd leaf extract has potential as an environmentally friendly and sustainable alternative to toxic chemical pesticides for controlling cabbage looper infestation.

Larvae of the 28-spotted potato ladybird (*Henosepilachna vigintioctomaculata*) can severely reduce potato (*Solanum tuberosum* L.) yields by feeding on the leaves. This study examined the effectiveness of an ethanol extract of black plum (*Syzygium cumini* L. Skeels) leaves was compared with that of the chemical pesticide permethrin 3.2 EC and aqueous dimethyl sulfoxide (control) for a late spring crop grown from May 19, 2025, to August 6, 2025, at the Field Science Center for Northern Biosphere, Hokkaido University (Sapporo, Japan). The experiment was laid out in a randomized complete block design where each treatment comprised three replications consisting of 30 plants in total. The black plum leaf extract showed high biopesticide activity against 28-spotted potato ladybirds and enhanced the potato yield by 14.06% compared with the control and 11.68% compared with permethrin. Application of the extract decreased 28-spotted potato ladybird infestation in field-grown potatoes by 71.43%, 52.94%, 47.62%, and 63.33% at 50 days after sowing (DAS), 58 DAS, 66 DAS, and 73 DAS, respectively, compared with the control. Two possible chemicals for biopesticides, acacetin-7-*O*-rutinoside and amorphigenin were identified by liquid chromatography–mass spectrometry analysis of the black plum leaf ethanol extract. The extract is a potential environmentally friendly, sustainable, and cost-effective biopesticide substitute for hazardous chemical pesticides for control of 28-spotted potato ladybirds.

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Chapter 1 General introduction

1.1. Background

1.1.1. Impact of pesticides used in agriculture

Sustainable agricultural systems demand less or no usage of synthetic chemical pesticide, which have various adverse effects on human health and environment. Pesticide exposure can occur at work, in places where pesticides are used, and through diet. The primary route of pesticide exposure for the general population is diet, particularly through pesticide residues on fruits, vegetables, and animal-derived food products (HBM4EU, 2022). Occupational exposure may arise when individuals in agricultural, forestry, or urban settings apply pesticides, maintain spraying equipment, or handle goods treated with pesticides. People who live close to fields where pesticides are applied may be exposed due to pesticide drift and volatilization, and agricultural workers have been observed to bring chemicals home on their clothing, exposing their families (Hernández *et al.*, 2019; Teysseire *et al.*, 2021).

Pesticides used in agriculture can have significant negative impacts on the environment, including soil and water pollution, harm to non-target organisms like beneficial insects and aquatic life, and potential contamination of food sources, posing risks to human health when residues are present; essentially, while pesticides can control pests, their widespread use can disrupt ecosystems and harm biodiversity if not managed carefully (Akter *et al.*, 2009).

Modern agriculture makes widespread use of pesticides, which are an efficient and cost-effective means of improving produce quality and quantity and guaranteeing food security for the world's expanding population. Globally, almost 3.73 million tons of pesticides are used each year, as of the latest data for 2023, Brazil is the largest consumer of agricultural pesticides by total mass, followed by the United States and Indonesia, which is growing quickly. However, it is predicted that the amount of pesticides used worldwide will rise to 4.4 million tons by 2027. Although pesticides are advantageous from the perspective of agricultural production, their biomagnification and persistent nature might have detrimental effects when used excessively. Numerous pesticides have either directly or indirectly contaminated the air, water, soil, and environment, posing a major threat to human health. Approximately one-third of agricultural goods are dependent on pesticide use. Without the use of pesticides, there would be a 78% decrease in fruit productivity, a 54% decrease in vegetable production, and a 32% decrease in cereal production. Globally, pesticides are essential for lowering disease rates and raising crop yields (Tudi, 2021). By deactivating or suppressing the activity of particular soil microbes that carry out vital biochemical activities in the soil, such as nitrification, ammonification, and nitrogen fixation, pesticides can interfere with these processes (Khan, 2023).

1.1.2. Development of biopesticide for sustainable food security

Biopesticides are certain types of pesticides derived from organic components like bacteria, plants, animals, and some minerals. Based on the kind of bioactive components or agents used for pest control, the USEPA has divided biopesticides into three main classes: (i) biochemical pesticides; (ii) microbial pesticides; and (iii) plant-incorporated protectants (EPA US, 2008).

In integrated pest management strategies, the rotation of crop protectants includes botanicals. With their antifungal, antiviral, and antibacterial properties, botanical extracts have the power to eradicate and discourage pests, prevent the growth and development of insects, prevent them from eating and reproducing, and provide protection against infections (Prakash and Rao, 1986). Plant extracts hindered insect growth and development, prolonged the time spent in the larval and pupal stages, reduced pupal recovery, and decreased adult eclosion. They significantly reduced the weight of insect larvae, pupae, and adults (Khanam *et al*., 1990). Additionally, adult emergence and larval survival rates are decreased by plant derivatives (Koul *et al*., 2008).

Bio-pesticides are equally effective as synthetic pesticides at controlling agricultural pests (Birech *et al*., 2006). They quickly biodegrade and do not affect the environment, natural products are also advantageous for the environment (Leng *et al.*, 2011). Bio-pesticides are acceptable alternatives to synthetic pesticides since customer tastes and preferences evolve over time and are impacted by the demand for food produced organically (Okunlola *et al*., 2014). Since they have a very short pre-harvest period, bio-pesticides are safe to use on fresh fruits and vegetables (Khater, 2012). Biopesticides are cheap, environment-friendly, specific in their mode of action, sustainable, do not leave residues, and are not associated with the emission of greenhouse gases (Ayilara, 2023). They support sustainable pest control, which supports sustainable agriculture, and are effective in little dosages (Nawaz *et al*., 2016). Natural insecticides don't cause pests to become resistant to them (Tadel *et al*., 2017). They are readily available and affordable to obtain because they may be found in the natural environment and some of them are used for other things, such as food and feed and their source materials (Srijita, 2015). The applicant's safety is ensured by the comparatively short re-entry intervals of natural pesticides (Stoneman, 2010). Additionally, bio-pesticides are used to purify agricultural soils by increasing the populations of beneficial microbes (Javaid *et al*., 2016).

1.1.3. Importance of botanical pesticides

Chemical pesticides are known to be extremely detrimental to both the environment and living things. Bio-pesticides are plant extracts, which are less hazardous to environment. On the other hand, a single type of botanical may only be helpful against one type of pest or insect, however, a mixture of two or more botanicals can help control all types of insects in a crop field (Azad *et al.,* 2012).

According to Dales (1996), the use of synthetic insecticides contaminates the environment and poses health concern. According to Schmutterer (2002), the World Health Organization (WHO) has documented that at least 3 million agricultural workers have been poisoned, with 20,000 deaths attributed to pesticide use per year. Approximately 385 million workers are affected by acute, unintentional pesticide poisoning annually worldwide. This means about 44% of the global population working in agriculture is poisoned every year (Cancino, 2023). Awasthi (2001) also noted that consumers of vegetables may be at risk from pesticide residues. Therefore, research has focused on finding non-chemical pest management techniques that are safe, affordable, simple to use, and available to farmers (Jilani and Su, 1983). Bio-pesticides are typically easy to prepare and apply at the farm level, have a broad spectrum of activity, and have a rather specific mode of action (Saxena *et al*., 1980). Plants are abundant in natural compounds that can be used to create environmentally friendly pest control techniques (Sadek, 2003).

Botanical pesticides, derived from plants, exert their effects on pests through various mechanisms, including repellency, inhibiting feeding, disrupting growth, and even causing direct mortality by interfering with essential metabolic processes or the nervous system (Lengai, 2020). Botanical pesticides also known as biopesticides or plant-derived pesticides, are natural products extracted from plants that are used to control pests, including insects, fungi, and other organisms, offering an alternative to synthetic chemical pesticides (Acheuk, 2022).

Research on current biological control agents and the creation of biopesticides was started in the middle of the 1970s by the World Health Organization and other international organizations. Chemical pesticides are universally acknowledged to be extremely detrimental to our ecosystem. People are searching for new pesticides that are safe to use in fields as a result. Chemical pesticides are created by a synthetic process in the chemical industry, whereas plant extracts are used to create bio-pesticides, which are anticipated to be less harmful to the environment. Application of bio-products is therefore the most important step to take to provide a better and safer environment without lowering crop production. Additionally, it has been challenging for most small-holder farmers to use these control techniques since they consistently lack the funds to purchase synthetic pesticides. As a result, they rely on a variety of pesticidal plants to control insect pests (Belmain and Stevenson, 2001; Ayilara, 2024).

1.2. Literature review

1.2.1. Devastation features of looper in cabbage and 28-spotted ladybird in potato

Cabbage is a genus of biennial plants that can be leafy green, crimson (purple), or white (pale green), and is grown as an annual vegetable crop for its densely leaved heads. They can be eaten raw, pickled, fermented, steamed, stewed, roasted, sautéed, or braised. A great source of fiber, vitamin K, and vitamin C is raw cabbage.

There are many kinds of pests including root-knot nematodes and cabbage maggots, aphids, harlequin cabbage bugs, thrips, striped flea beetles, diamondback moth, cabbage white butterfly and caterpillars (Bradley, 2009). In North America, the cabbage looper (*Trichoplusia ni*) is notorious for its ravenous eating and for contaminating plants with frass (Turini, 2020). The cabbage looper (*Trichoplusia ni*) is a medium-sized moth in the family Noctuidae, a family commonly referred to as owlet moths. Its common name comes from its preferred host plants and distinctive crawling behavior. Cruciferous vegetables, such as cabbage, bok choy, and broccoli, are their main host plant; hence, the reference to cabbage in its common name. Because the larvae arch its back into a loop while crawling, it is known as a looper (Capinera, 2001). The eggs of cabbage loopers are typically dome-shaped, ridge-patterned, and yellow white in appearance. In a single day, 40–50 females can lay 1000–2000 viable eggs. After roughly three days, viable eggs begin to hatch, whereas unviable eggs fail to develop and eventually collapse. One kind of cabbage worm that is green in color with a white stripe on the side is called a cabbage looper larva. They are green and slightly hairy when they first hatch, but they ultimately turn green and shed the hair, leaving only a few bristles. When they crawl, they arch their bodies in a loop, which is how they recognize. Larvae can have four to seven instars in nine to fourteen days, and they are typically three to four centimeters long. The life cycle of a cabbage looper typically lasts 24 to 33 days from egg to adulthood (Jennifer, *et al.* 2005; McEwen and Hervey, 1960; Wilson, *et al.,* 1982). Pesticides used to treat cabbage: Chlorpyrifos, Cypermethrin, Chlorfluazuron, Cyantraniliprole, *Bacillus thuringiensis*, Chlorantraniliprole, Spinetoram, Chlorothalonil, Dimethoate, Cyhalothrin, Deltamethrin (Weinberger & Srinivasan, 2009; Zhang *et al.,* 2007). Experimental cabbage field of this research is shown in Figure 1.1.

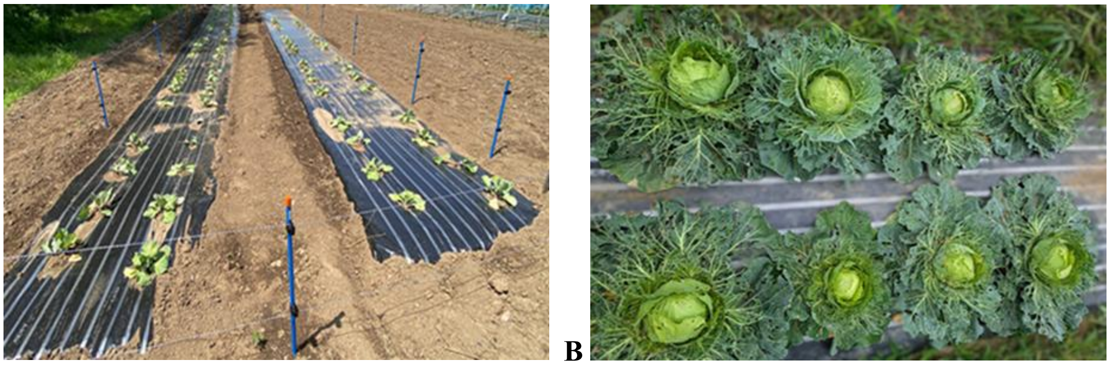
A  B

Figure 1.1 (A) Experimental cabbage field and (B) infected cabbage plot

Potato is the 4th most important food crop in the world after rice, wheat and maize. In many nations, potatoes are a staple food and the largest non-cereal food crop (Zhang, 2017). It is the cheapest type of carbohydrate and provides a considerable amount of vitamin B6, C, and several minerals (Singha, and Maezawa, 2019; King and Slavin, 2013). A large quantity of potatoes is used as raw materials in the manufacturing of alcohol, glucose, dextrin, and citric acid for the textile and paper industries, as well as inks, dyes, soap, and leather (Abouzied and Reddy, 1986).

Major insect pests of potato include the Colorado potato beetle, 28-spotted potato ladybird beetle, potato tuber moth, cutworms, white grubs, leafhoppers, thrips, and flea beetles and aphids, which can cause significant yield loss through leaf and tuber damage, or by transmitting diseases. Plants in the Solanaceae family suffer significant harm by the 28-spotted potato ladybird, *Henosepilachna vigintioctomaculata*. It is a phytophagous (plant-eating) species that feeds on the leaves of plants using its chewing mouthparts. Nowadays the primary strategy for shielding crops from the potato ladybird beetle is the use of pesticides. In addition to eliminating the pest, this results in the contamination of agricultural environments and the rise of pesticide-resistant potato ladybird populations. Its tiny egg masses typically hold 15–30 eggs, however they can occasionally hold 50–70 eggs. 200–500 eggs can often be laid by a single female. The undersides of potato leaves or weeds are the main places where the potato ladybird beetle lays its eggs. It causes significant devastation to potato crops by feeding on the leaves in both its adult and larval stages, which can lead to complete defoliation and total crop failure in severe cases (Simakova, 1978). Pesticides used to treat potatoes are: methamidophos, pymetrozine, pirimicarb, and spirotetramat etc (Kanario, *et al.* 2025).

Therefore, an alternative eco-friendly way of eliminating insect pests of potato is most desired in the world. Botanical extracts are the safer substitute for chemical fungicides among the several alternative materials that are accessible. Several antimicrobial metabolites found in botanical extracts are less harmful to people and the environment. Experimental potato field of this research is shown in Figure 1.2.

A  B 

Figure 1.2 Experimental potato field: (A) seed sowing (B) plants after 30 DAS (Days after sowing)

1.2.2. Development of biopesticide from bottle gourd leaves and black plum leaves

More of nature is dominated by the plant world. Increased waves of interest have been seen in the study of natural product chemistry. Unmet medicinal demands, the astounding variety of chemical structures and biological functions of naturally occurring secondary metabolites, and the usefulness of novel bioactive natural chemicals as biochemical probes are a few factors that can be attributed to this phase. Gas chromatography-mass spectroscopy technique to analyze the phytochemical components in hexane and methanol extract of bottle gourd (*Lagenaria siceraria*) leaves respectively are given Table 1.1 and Table 1.2 (Aldewya, 2022; Sultana *et al.*,2024).

Table 1.1 Identified fourteen compounds (hexane soluble) from air-dried leaves of bottle gourd by GC-MS.

Compound Name	MF	Rt(min)	MW
Caryophyllene	$C_{15}H_{24}$	11.91	204
Neophytadiene	$C_{20}H_{38}$	18.94	278
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H4_{0}O$	19.36	296
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	20.48	904
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	21.81	256
(Z,Z)-9,12-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	23.08	256
(Z,Z,Z)-9,12,15-Octadecatrienoic acid methyl ester	$C_{19}H_{32}O_2$	23.20	292
Phytol	$C_{20}H4_{0}O$	23.41	296
Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	23.59	298
(Z,Z)-9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	24.38	280
(Z,Z,Z)-8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	24.67	306
Di-n-octyl phthalate	$C_{24}H_{38}O_4$	29.33	390
Stigmast-5-en-3-ol	$C_{29}H_{50}O$	35.76	414
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	36.78	436

Table 1.2 Thirteen (methanol-soluble) compounds detected in bottle gourd leaf extract by GC-MS.

Name of the compounds	Molecular formula	Retention time	Molecular weight (g/mol)	% of composition
1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z, E)-	$C_{15}H_{24}$	14.37	204.35	2.557
Hexadecanal	$C_{16}H_{32}O$	22.78	240.43	2.109
Dodecane, 1,1-dimethoxy-	$C_{14}H_{30}O_2$	26.991	230.39	6.363
Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	28.21	284.48	4.346
Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	$C_{20}H_{38}O_2$	31.93	310.5	1.293
Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	12.33	206.24	16.666
Pentadecanoic acid, 14-methyl-, methyl ester	$C_{16}H_{32}O_2$	28.22	270.45	2.748
1,2-Benzenedicarboxylic acid, butyl 2-ethylhe	$C_{20}H_{30}O_4$	28.83	334.45	6.532
9,12-Octadecadienoic acid, methyl ester, (E, E)-	$C_{19}H_{34}O_2$	31.80	294.47	17.713
9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	31.95	296.49	4.697
Undecanoic acid, 10-methyl-, methyl ester	$C_{13}H_{26}O_2$	28.20	214.34	6.294
Phytol	$C_{20}H_{40}O$	32.13	296.54	12.015
1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	26.55	278.34	16.667

29 Phytoconstituents identified from methanolic extract of black plum leaves by (GC-MS) (Mohareb, 2023). Vivek identified some phytoconstituents from methanolic extract of *Syzygium cumini* leaves are given Table 1.3 (Vivek, 2021).

Table 1.3 Phytoconstituents identified from methanolic extract of *Syzygium cumini* leaves.

RT	Name of Compound	Molecular Formula	Molecular Weight (gm/mol)	Peak Area %
2.31	(1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	136.23	7.54
4.42	2,5,5-trimethyl-cyclopentadiene	C_8H_{12}	108.18	1.87
8.27	α-terpineol	$C_{10}H_{18}O$	154.25	1.95
12.03	1,2,3-benzenetriol	$C_6H_6O_3$	126.11	9.24
12.50	1,4-methanocycloocta[d]pyridazine,1,4,4a,5,6,9,10,10a-octa hydro-11,11-dimethyl-(1.α,4.α,4a.α,10a.α)	$C_{13}H_{20}N_2$	204.15	1.2
28.90	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	7.28
29.45	9,12,15-octadecatrienoic acid, methyl ester (Z, Z, Z)	$C_{19}H_{32}O_2$	292.46	10.45
48.68	Vitamin E	$C_{29}H_{50}O_2$	430.71	13.23
51.89	γ-sitosterol	$C_{29}H_{50}O$	414.71	34.01

1.3. Hypothesis and main objectives of this study

According to recent data, global pesticide use has been steadily increasing in recent years, nearly doubling since 1990, with the largest increases seen in low-income countries; this rise is attributed to factors like growing food demands and the need for crop protection against pests, although concerns remain regarding environmental and health impacts from widespread pesticide application. In 2022, the consumption volume of insecticides in Japan amounted to about 14.75 thousand metric tons (Shattuck, 2023). Bottle gourd leaves and black plum leaves may have many bioactive compounds which can control cabbage looper and 28-spotted potato ladybirds respectively, reduce pollution and protect the human health and environment. The present study has been undertaken to fulfill the following objectives-

I. To determine the efficacy of bottle gourd leaf and black plum leaf extract in controlling cabbage looper and 28-spotted lady bird on field-grown cabbage and potato, respectively.

II. To determine the effect of the extracts on the growth and yield of cabbage and potato.

III. To analyze the biopesticide compounds of the bottle gourd leaf and black plum leaf.

Chapter 2 Efficacy of bottle gourd (*Lagenaria siceraria*) leaf extract in protecting against cabbage looper (*Trichoplusia ni)* (Lepidoptera: Noctuidae) infestation and LC–MS analysis

2.1. Introduction

The use of synthetic chemical pesticides, which have various adverse effects on human health and the environment must be reduced or prohibited to establish sustainable agricultural systems. Synthetic pesticides are dangerous when used frequently because they disrupt natural predators, pollinators, and other wildlife; contaminate groundwater; promote the development of resistance; and may cause the spread of secondary pests (Dubey *et al.*, 2010).

As ecofriendly biopesticides do not pollute the air, water, or soil with harmful chemicals, they are safer for the environment and human health. Botanicals are plant-derived substances that act by a variety of mechanisms (Weaver *et al.*, 2000). These substances are derived from either fresh or dried plant materials, such as leaves, bark, flowers, roots, rhizomes, bulbs, seeds, cloves, or fruit (Chougule and Andoji, 2016). When applied at the optimal time, concentration, and frequency, biopesticides can outperform synthetic pesticides (Shah *et al.*, 2013). Because biopesticides have a very short preharvest withholding period, they are safe for use on fresh fruits and vegetables (Khater, 2012).

Cabbage (*Brassica oleracea*) is a leafy green biennial plant known for its tightly packed heads of leaves and is a member of the Brassicaceae family (Richardson, 2016). Among the most widely used food crops, cabbage thrives in many regions of the world (Embaby and Lotfy, 2015). In 2022, total cabbage production in Japan was 1,458,200 Mt, according to Japan’s Ministry of Agriculture, Forestry and Fisheries. Cabbage plants can be infested by many types of insects, such as cabbage looper, aphids, large white butterfly, diamondback moth, cabbage moth, cabbage maggots, thrips, and striped flea beetles, depending on the variety of cabbage, weather, and region. During the present experiment, only the cabbage looper (*Trichoplusia ni*) was visible from the seedling stage to the final growth stage. Other pests were present in extremely minor quantities and thus were not taken into consideration.

The cabbage looper larva is a type of cabbage worm, which is a general term for insects in the Lepidoptera order that primarily feed on cruciferous vegetables. The cabbage looper is most problematic once the plant enters the heading stage. The larvae eat the underside of the leaves and consume the developing head. The infamous reputation of this pest likely stems from its ability to infest a variety of crops readily and the difficulty in managing the pest (Charles *et al.*, 1958). Eggs are deposited singly or in small clusters on either leaf surface, although more eggs are found on the lower leaf surface. Each female moth can produce 300–600 eggs within approximately 10–12 days. After the eggs hatch, additional larvae move to the lower leaf surface to feed. Two to four weeks after hatching, the mature larva forms a thin cocoon on the lower leaf surface or in plant debris or soil. The pupal stage lasts approximately two weeks. With warm temperatures, the development of the cabbage looper, from egg to adult, takes approximately 18 to 25 days (Elwakil and Mossler, 2013).

Bottle gourd (*Lagenaria siceraria*), also called white-flowered gourd or calabash gourd, is a running or climbing vine of the gourd family (Cucurbitaceae). Bottle gourd was among the first plants to be domesticated and the only one with a global distribution during pre-Columbian times (Kistler *et al.*, 2014). Bottle gourd leaf extracts, at 10% and 20% concentrations, have shown good efficacy in reducing aphid populations on cabbage plants (Sultana *et al.*, 2024). Islam et al. reported that a water gourd leaf extract showed good efficacy against aphids in a cabbage field experiment (Islam *et al.*, 2020). A petroleum ether extract from the leaves of *L. siceraria* provided maximum protection (100%) against *Culex pipiens* larvae (Hassan *et al.*, 2014). Thus, bottle gourd leaf extracts may be useful as biopesticides in cabbage fields instead of chemical pesticides to control cabbage looper infestation and reduce environmental pollution. Therefore, the present study was conducted to determine the efficacy of a bottle gourd leaf extract in controlling cabbage looper growth in field-grown cabbage, to determine the phytochemical effects of the extract on the growth and yield of cabbage, and to analyze the phytochemical composition of the bottle gourd leaf.

2.2. Materials and methods

2.2.1. Experimental site and plot preparation

This experiment was conducted in the late spring crop season from April 15, 2024, to August 20, 2024, at the Field Science Center for the Northern Biosphere, Hokkaido University, Hokkaido, Japan. The experimental site is located at 43°4′ N latitude and 141°20′ E longitude at an altitude of 20 m above sea level. The weather conditions from seedling to harvest are listed in table S1, and the characteristics of the soil properties present in the cultivation conditions are listed in table S2. The experiment was carried out in a randomized complete block design, with each treatment comprising three replications consisting of 24 plants in total. The unit plot size was 1.2 m × 1.8 m, the distance between the blocks (i.e., between replications) was 0.75 m, the spacing between individual plants was 0.45 m, and the spacing between rows was 0.5 m. The soil was prepared for sowing by plowing it five times followed by laddering. The crop stubble and uprooted weeds were removed from the soil, and the land was leveled before the cabbage seedlings were planted. Before the seedlings were transplanted, 25 g of organic fertilizer was incorporated into every planting hole.

The determinate cabbage variety used in this experiment, ‘Kinkei-201’, is popular with local growers and was purchased from Snow Brand Seed Co., Ltd. (Sapporo, Hokkaido, Japan). At the four-leaf stage, uniform healthy seedlings were transplanted into the experimental plots. The field was irrigated a total of 12 times during the growing season. Field management for protection from small animals was achieved by installing two area pole insulators Ø 20AP-PL900GB, an Apollo Solar Electric Fence Area System body AP-2011-SR, and a 500 m orange Shinsei electric fence rope.

2.2.2 Preparation of treatment and control solutions

Bottle gourd leaves were collected from the campus of Hokkaido University, Japan, as a source of plant materials with potential biopesticide properties. The samples were first visually examined for any type of infection, spores, damage, discoloration, or distortion. Undamaged leaf samples were thoroughly washed with running tap water and then allowed to dry in ambient light at room temperature. The dried leaves were ground to powder with a high-speed grinder (WB-1, Osaka Chemical Co., Ltd., Osaka, Japan). Approximately 100 g of the powder was placed in a conical flask, and 400 ml of ethanol was added. The conical flasks were shaken in a constant-temperature incubator shaker (Bioshaker BR-41FL, TAITEC Co., Koshigaya City, Saitama, Japan) for 74 h at 120 rpm. After centrifugation (Tomy MX-305 high-speed refrigerated microcentrifuge, Tomy Kogyo Co., Ltd., Fukushima, Japan) for 20 min at 2075 ×*g*, the extract was filtered through Whatman No. 1 filter paper. The filtrate was placed in an EYELA-type constant-temperature air-drying oven (WFO-420 W, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 50 °C to evaporate the ethanol. Then, 1 g of crude extract from the bottle gourd leaves was dissolved in 1 ml of DMSO and added to 1000 ml of distilled water (Figure 2.1). The trial testing the efficacy of the crude extract also included a control (0.1% [v/v] aqueous DMSO) and a chemical insecticide treatment, permethrin (3.2 emulsifiable concentrate), as the positive control. The positive control, permethrin (3.2 EC formulation), was applied at a rate of 10 mL per 1 L of water (1% [v/v] aqueous solution). Concentration of the treatment and control solutions are shown in table 2.1. Permethrin is a widely used pesticide owing to its effectiveness against insects and its low toxicity to mammals. Permethrin acts on the nerve cell membrane to disrupt the sodium channel current. Delayed repolarization and paralysis of pests result from this disturbance (Permethrin, 2024). DMSO $(CH_3)_2SO$ is a powerful solvent for a wide range of organic and inorganic compounds, is suitable for diluting plant extracts that may contain a mixture of different compounds, and concentrations of less than 0.1% are safe for plants and animals (Gallardo-Villagrán *et al.*, 2022).

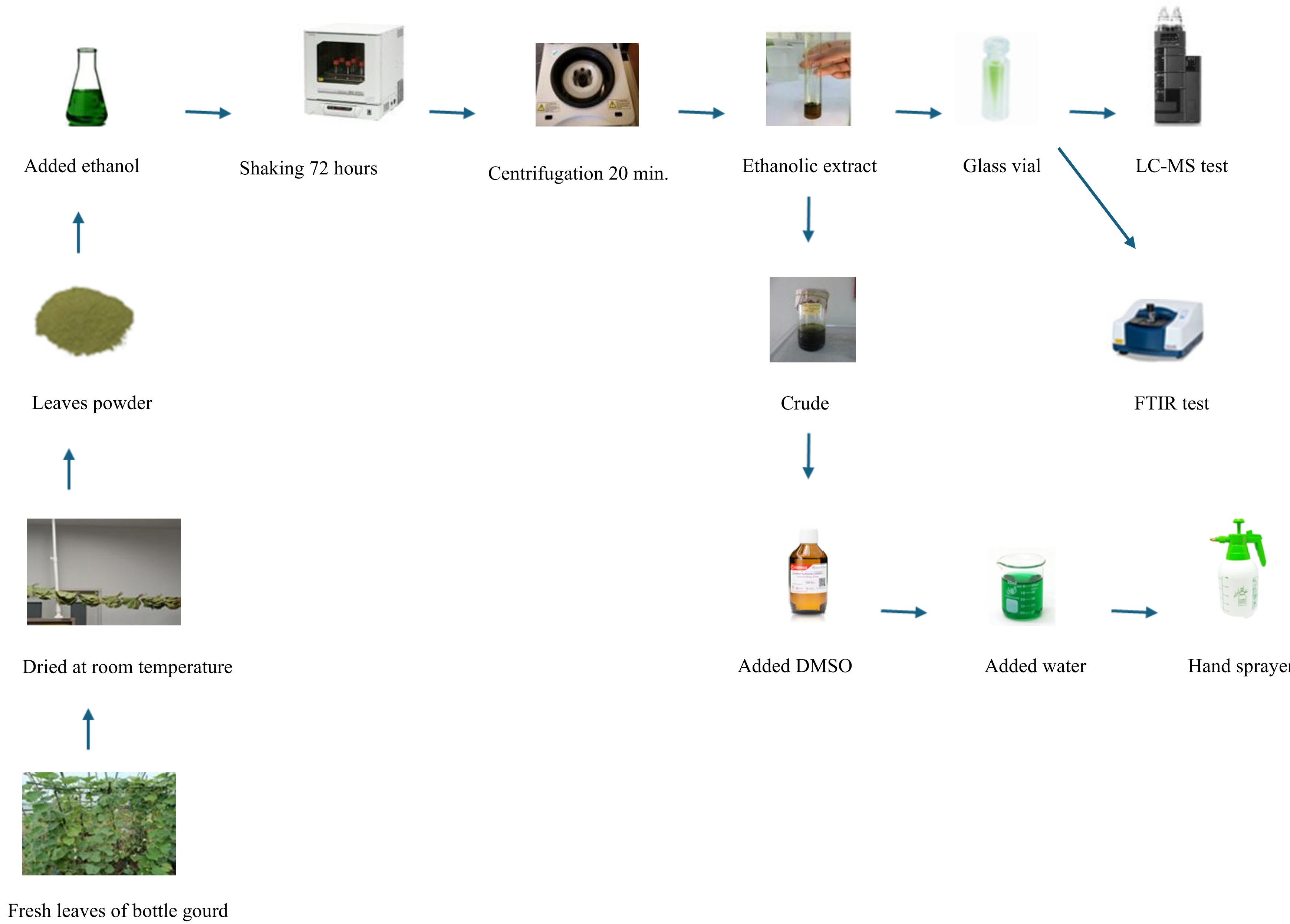


Figure 2.1 Procedure of bottle gourd leaves extract preparation

Table 2.1 Concentrations of the treatment and control solutions

Treatments	Chemical pesticide (Permethrin 3.2 EC)	Bottle gourd leaves	Control (aqueous DMSO)
Concentrations	1%	0.1%	0.1%

2.2.3. Spray application of solutions

The prepared solutions were sprayed onto the cabbage seedlings in the experimental field with a hand-held garden sprayer (capacity 1.5 L; Taizhou City Hangyu Plastic Co., Ltd., Zhejiang, China) in the morning (09:00) at 7-day intervals (Figure 2.2). The spray applications were initiated at 10 days after transplanting (DAT) the cabbage seedlings and continued until the end of the growing season (58 DAT). During this growing period, cabbage plants were sprayed eight times in total with the treatments and the control at a volume of 10 ml per cabbage plant per application.

Figure 2.2 A. Cabbage looper larvae B. infected plant C. spraying

2.2.4. Looper infestation monitoring and growth indicator measurement

Cabbage looper infestations on the cabbage plants were monitored from the start of insect colonization until the cabbage plants reached maturity. First, the number of leaf perforations on all infested leaves was counted with the naked eye. The number of insects was counted using a per-leaf sampling system (Church and Strickland, 1954). The plots were sampled daily by visual inspection of both leaf surfaces in the morning for 7 weeks from July 10 to August 30. Cabbage looper larvae within the canopy were counted for eight cabbage plants in each plot. These larvae were collected by hand from each treatment plot and placed in a plastic bag for disposal. Plant growth indicators were measured in the field in August 2024, and yield data were collected after harvesting at 70 DAT. The plant height and stem diameter (cm) were measured, and the SPAD value was determined to estimate the leaf chlorophyll content, which was measured with a portable chlorophyll meter (SPAD-502, Minolta Camera Co., Ltd., Osaka, Japan). The photosynthesis rate was measured with a plant photosynthesis meter (miniPPM-300, EARS, Delft, The Netherlands). The total cabbage yield (kg/plot) was recorded, and the percentage yield increase over that of the control was calculated using Eq. 1 (Islam *et al.*, 2021):

$$\text{Yield increase or decrease (\%)} = \frac{\text{Treatment yield} - \text{Control yield}}{\text{Control yield}} \times 100. \qquad \text{(Eq. 1)}$$

The percentage cabbage looper infestation (%) of the bottle gourd leaf extract group was calculated using Eq. 2:

$$\text{Cabbage looper infestation (\%)} = \frac{\text{Looper number in bottle gourd leaf extract group} - \text{Looper number in Control group}}{\text{Looper number in Control group}} \times 100. \qquad \text{(Eq. 2)}$$

2.2.5. Sample preparation for phytochemical analysis

Bottle gourd leaves extract preparation was done by following the standard procedures described previously. All chemicals and solvents used for LC–MS were HPLC grade.

2.2.5.1. Liquid chromatography–mass spectrometry analysis

Liquid chromatography–mass spectrometry (LC–MS) analysis of the ethanolic extract of bottle gourd leaves was performed using a Nexera-XR liquid chromatograph (Shimadzu, Kyoto, Japan) coupled with a mass spectrometer (LTQ-Orbitrap XL, Thermo Fisher Scientific, Waltham, MA, USA). The extract (5 µL) was injected into an Inert Sustain AQ-C18 column (1.9 µm, 2.1 × 100 mm; GL Sciences, Tokyo, Japan) maintained at 40 °C. Aqueous formic acid (0.1%, solvent A) and 0.1% formic acid in acetonitrile (solvent B) were used as the mobile phases. Gradient elution was carried out at 150 µL/min using the following software: 50% B for 0 min, 95% B for 15 min, 95% B for 20 min, 50% B for 21 min, and 50% B for 26 min.

Electrospray ionization was used in positive mode. Other instrument settings were as follows: source voltage, 4.0 kV; nitrogen sheath gas flow rate, 30 L/min; auxiliary gas flow rate, 10 L/min; sweep gas flow rate, 0 L/min; capillary temperature, 300 °C; capillary voltage, 20 V; and tube lens voltage, 80 V. All extracts were evaluated in Fourier transform mass spectrometry (FTMS) mode, and the mass range was acquired by full-range acquisition covering *m*/*z* 100–2000. The samples were typically diluted (1:200) with solvent A. Data analysis was performed using XCalibur software v2.0.7 (Thermo Fisher Scientific).

2.2.5.2. Fourier transform infrared spectroscopy analysis

The functional groups and molecular structures on the surface of the ethanolic extracts of bottle gourd leaves were examined using Fourier transform infrared spectroscopy (FTIR) (FT/IR-4X, JASCO Corporation, Tokyo, Japan). Each spectrum was produced by averaging 50 scans in the range of 500−4000 cm^{-1} with a resolution of 4 cm^{-1}. The baselines were adjusted for atmospheric CO_2 and water vapor to ensure accurate readings.

2.2.6. Statistical analysis

The biopesticide activity of bottle gourd leaf extract against cabbage looper larvae on field-grown cabbage plants was tested in an experimental plot using a randomized complete block design. The data were entered into Excel (Microsoft) and subjected to one-way analysis of variance. Significant differences among treatments were detected using Duncan's multiple range test ($P < 0.05$) using IBM SPSS Statistics v.20 software (IBM Corp., Armonk, NY, USA) (Duncan, 1951).

2.3. Results

2.3.1. Effects of ethanolic extracts of bottle gourd leaves on the average number of cabbage looper larvae per plant at the fifth, sixth, seventh, and eighth weeks

The chemical pesticide permethrin provided the strongest protection against cabbage looper larvae (Figure 2.3). Compared with the control (aqueous DMSO), the bottle gourd leaf extract had good efficacy against cabbage looper larvae. Compared with the control (aqueous DMSO), the application of the extract significantly decreased looper larval infestation by 41.18%, 39.71%, 52.08%, and 37.96% at the fifth, sixth, seventh, and eighth weeks, respectively, in field-grown cabbage. The greatest infestation of cabbage looper larvae was observed at the sixth week, whereas the infestation at the fifth and seventh weeks was low, and after the eighth week, no larvae were observed.

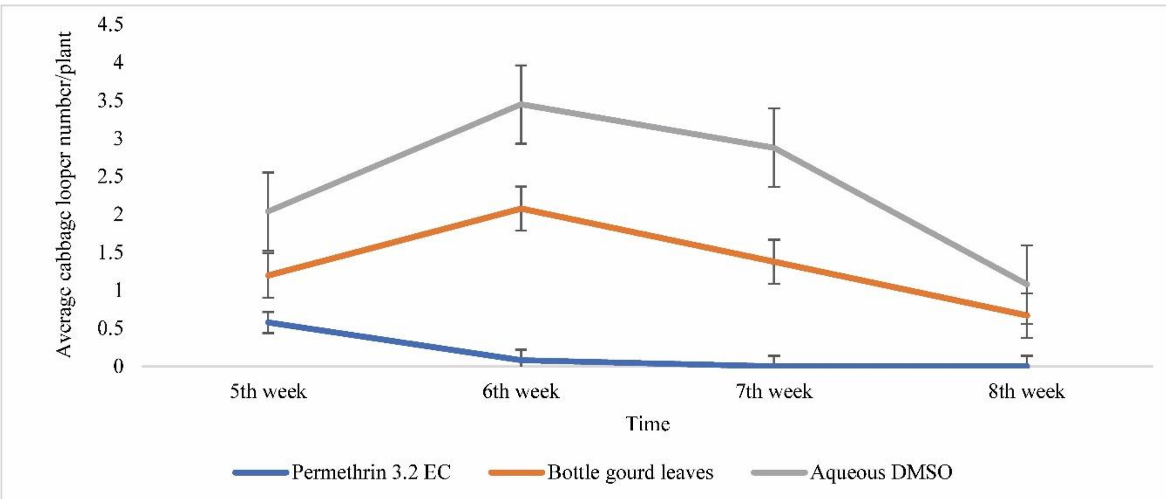


Figure 2.3 Biopesticide activity of the ethanolic extract of bottle gourd leaves against cabbage looper (*Trichoplusia ni*) larvae relative to that of permethrin and aqueous DMSO. The error bars represent the standard errors ($P < 0.05$; n = 24).

2.3.2. Effects of the ethanolic extract of bottle gourd leaves on the total number of leaves per plant, number of infested leaves, and average number of perforations per infested leaf at 30, 40, 50, and 60 DAT

Cabbage plants in the treatment groups and the control group produced very similar total numbers of leaves (Figure 2.4 A). The number of infested leaves was very low in response to the chemical pesticide treatment, whereas the bottle gourd leaf extract had a moderate effect compared with that of the control (Figure 2.4 B). The number of perforations in infested leaves of cabbage plants treated with the bottle gourd leaf extract was moderate at 40 and 50 DAT, but at 60 DAT, the number of perforations was similarly reduced by the bottle gourd leaf extract and the chemical pesticide compared with the control (Figure 2.4 C).

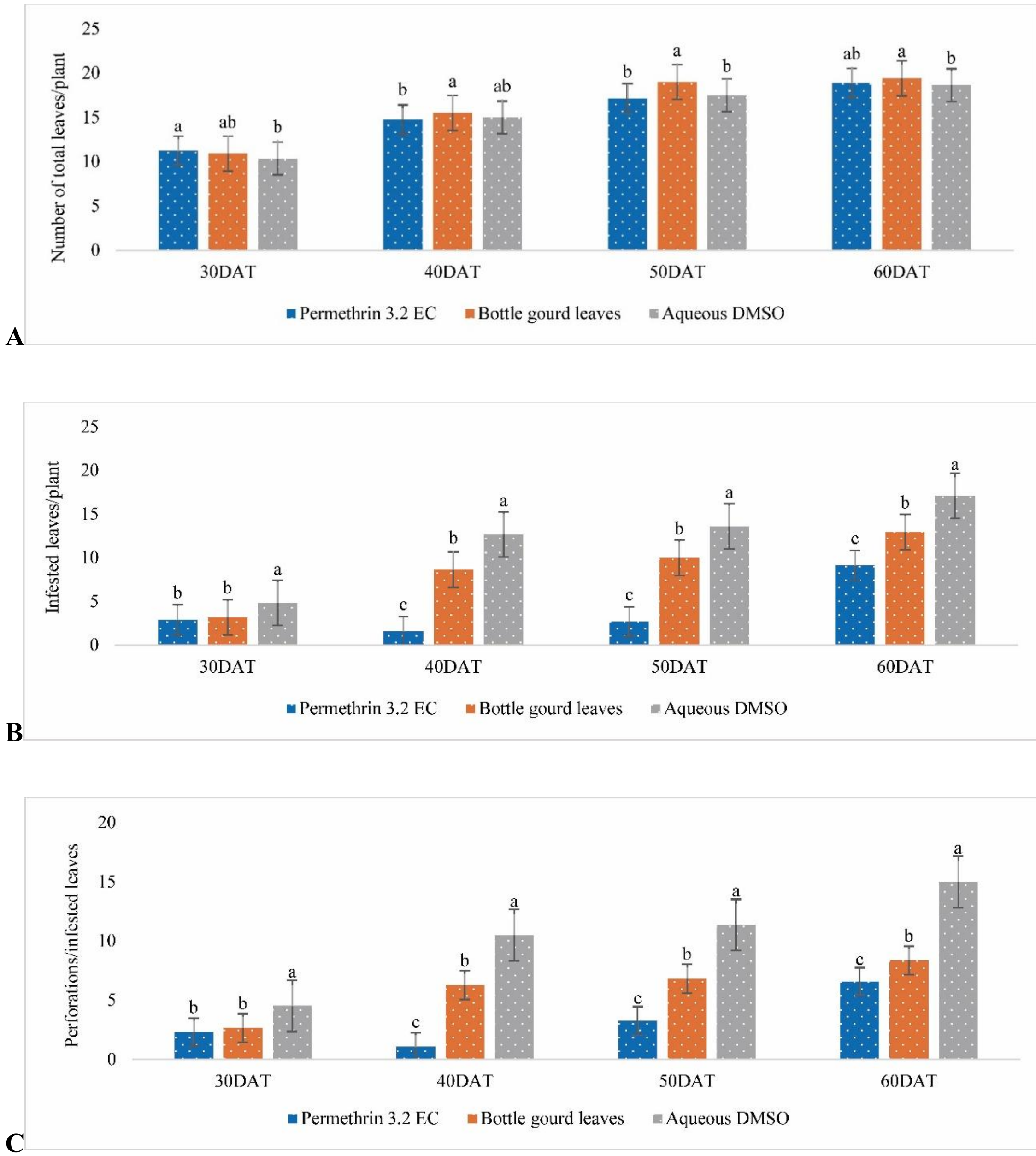


Figure 2.4 Effects of the ethanolic extract of bottle gourd leaves on the total number of leaves per plant; B infested leaves, and C average perforations per infested leaf of cabbage at 30, 40, 50, and 60 days after treatment (DAT). Different lowercase letters above the bars indicate significant differences as assessed by Duncan's multiple range test, and the error bars represent the standard error (*P* < 0.05; n = 24).

2.3.3. Effects of the ethanolic extract of bottle gourd leaves on the relative chlorophyll content (SPAD), photosynthesis rate, and plant growth indicators

No significant difference in the relative chlorophyll content (SPAD) or photosynthesis rate was detected between the permethrin 3.2 EC and bottle gourd leaf extract treatments and the control (Figure 2.5 A and 2.5 B). Compared with the chemical pesticide and control groups, the group treated with the bottle gourd leaf extract presented the greatest plant height, although the difference was not significant (Figure 2.5 C). No significant differences in plant spread (cm) or head diameter (cm) were detected among the permethrin 3.2 EC and bottle gourd leaf extract treatments compared with the control (Figure 2.5 D and 2.5 E).

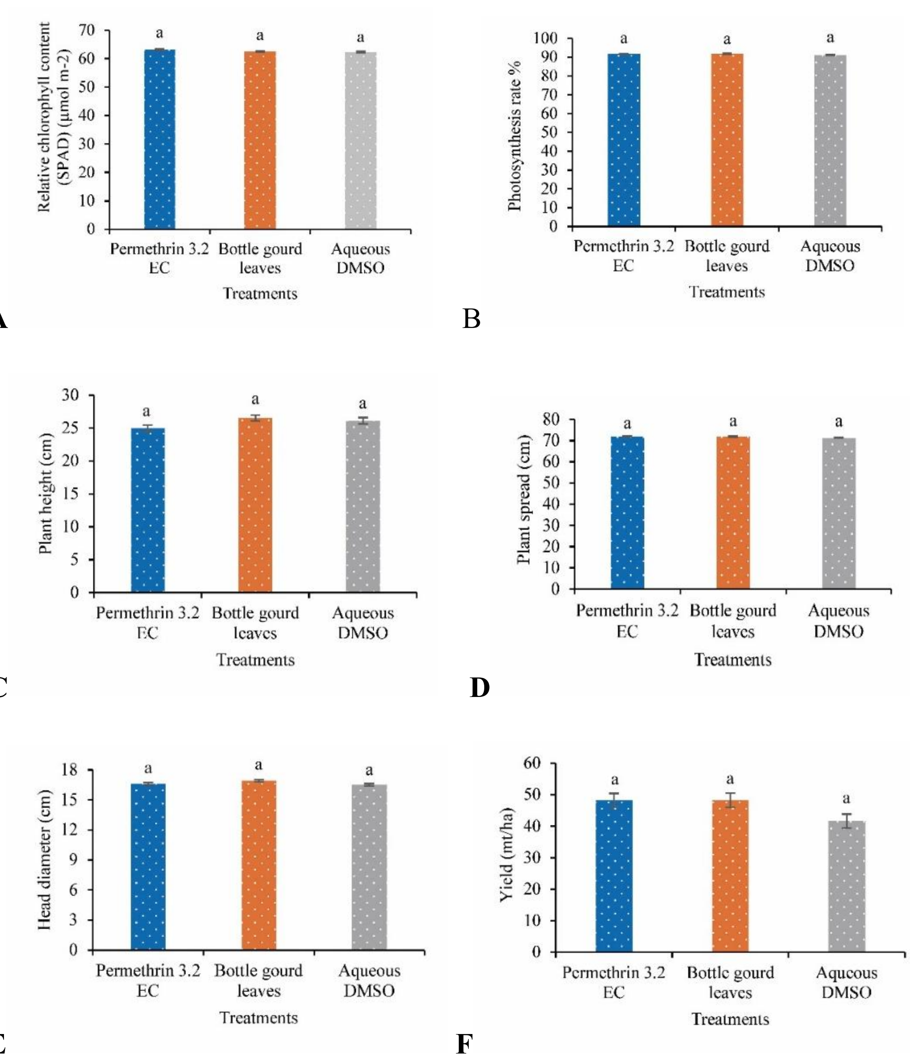


Figure 2.5 Effects of the ethanolic extract of bottle gourd leaves on the A relative chlorophyll content (SPAD); B photosynthesis rate; C plant height; D plant spread; E head diameter; and F yield. Different lowercase letters above the bars indicate significant differences as assessed by Duncan’s multiple range test, and error bars represent standard errors ($P < 0.05$; $n = 24$).

2.3.4. Effects of the ethanolic extract of bottle gourd leaves on cabbage yield

The chemical pesticide and bottle gourd leaf extract treatments produced yields of 48.23 and 48.26 mt/ha cabbage, respectively, and provided good protection from cabbage looper larvae. In contrast, the control (aqueous DMSO) produced 41.61 mt/ha, and the efficacy against looper infestation was very poor. The bottle gourd leaf extract showed high biopesticide activity against cabbage looper infestation and increased the cabbage yield by 15.98% compared with that of the control (aqueous DMSO), which was almost equal to that of the permethrin 3.2 EC-treated group (Figure 2.5 F).

2.3.5. Characterization and identification of biopesticide compounds in the ethanolic extract of bottle gourd leaves by LC–MS analysis

The LC–MS analysis of the ethanolic extract of bottle gourd leaves enabled the identification of 21 chemical compounds (Figure 2.6): apigenin-7-*O*-glucoside; 4-isopropyl-2-nitrophenol; 1-myristoyllysophosphatidic acid; (2,2-dichloro-1-(4-methyl-benzoylamino)-vinyl)- phosphonic acid diethyl ester; pentafluorobenzoic acid, 2-amino-9H-pyrido[2,3-b]indole; succinic acid, 2,2,3,3,4,4,5,5-octafluoropentyl pentafluorobenzyl ester; lyalosidic acid; indole-3-butyric acid; 5- (3,4,5-trimethoxybenzoyl)-2,4-pyrimidinediamine; phenol, 2-methoxy-4-(1-propenyl)-, acetate, (Z)-; strychnine; cytosine, N,N'-di(pentafluoropropionyl)-; phytol; 2-methyl-1,4-butanediol, bis(heptafluorobutyrate); 3-hydroxyechinenone; 7-acetyl-1,dione;(1Z)-1-[(3,4-diethoxyphenyl)methylidene]-6,7-diethoxy-3,4-dihydro-2H-isoquinoline; PE 34:3; hexadecanoic acid; luteolin 7-glucuronide-3',4'-dirhamnoside; ciprofloxacin and 2,6-dimethylquinoline. Basic information on each component of the bottle gourd leaf extract is presented in Table 2.2.

Table 2.2 Tentatively identified compounds in the ethanolic extract of bottle gourd leaves and their respective retention times (R/T), chemical names, elemental formulas, detected m/z values, and exact m/z values

R/T	Proposed compounds	Elemental formula	Class	Detected *m/z*	Exact *m/z*	% of Conc.
1.85	Apigenin-7-*O*-glucoside	$C_{21}H_{20}O_{10}$	flavonoid	433.11	432.11	8.65
2.13	4-isopropyl-2-nitrophenol	$C_9H_{11}NO_3$	phenol	182.99	181.00	3.24
2.99	1-Myristoyllyso phosphatidic acid	$C_{17}H_{35}O_7P$	lipid	383.15	382.41	3.24
5.74	(2,2-Dichloro-1-(4-methyl-benzoylamino)-vinyl)-Phosphonic acid diethyl ester	$C_{14}H_{18}Cl_2NO_4P$	Phosphor thioic acid	367.15	366.18	9.73
6.76	Pentafluorobenzoic acid, pent-2-en-4-ynyl ester	$C_{12}H_5F_5O_2$	Organo fluorine	277.22	276.16	4.87
7.53	2-Amino-9H-pyrido[2,3-b]indole	$C_{11}H_9N_3$	Hetero cyclic aromatic amines	184.99	183.21	10.81
8.57	Succinic acid, 2,2,3,3,4,4,5,5-octafluoropentyl pentafluorobenzyl ester	$C_{16}H_9F_{13}O_4$	fluorinated ester	513.25	512.22	2.16
8.74	Indolebutyric acid	$C_{12}H_{13}NO_2$	auxins	205.20	203.24	8.65

10.05	5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	$C_{14}H_{16}N_4O_4$	Hetero cyclic amine	305.23	304.12	1.08
10.55	Phenol, 2-methoxy-4-(1-propenyl)-, acetate, (Z)-	$C_{12}H_{14}O_3$	phenol	207.10	206.24	10.27
10.75	Strychnine	$C_{21}H_{22}N_2O_2$	alkaloid	335.15	334.17	2.16
12.69	Cytosine, N,N'-di(pentafluoropropionyl)-	$C_{10}H_3F_{10}N_3O_3$	pyrimidne	404.32	403.13	1.08
13.25	Phytol	$C_{20}H_{40}O$	alcohol	297.32	296.53	10.27
15.3	2-Methyl-1,4-butanediol, bis(heptafluorobutyrate)	$C_{13}H_{10}F_{14}O_4$	alcohol	497.26	496.19	1.08
15.35	3-Hydroxyechinenone	$C_{40}H_{54}O_2$	terpenoid	568.43	566.9	1.08
15.70	7-Acetyl-1,3-dimethylpurine-2,6-dione;(1Z)-1-[(3,4-diethoxyphenyl)methylidene]-6,7-diethoxy-3,4-dihydro-2H-isoquinoline	$C_{33}H_{41}N_5O_7$	alkaloid	621.31	619.7	6.49
17.56	PE 34:3	$C_{39}H_{72}NO_8P$	lipid	714.55	713.50	1.08
17.75	Hexadecanoic acid	$C_{18}H_{36}O_2$	fatty acid	285.28	284.48	10.27
20.82	Luteolin 7-glucuronide-3',4'-dirhamnoside	$C_{33}H_{38}O_{20}$	flavonoid	756.56	754.7	0.54
23.27	Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	Fluoro quinoloe	332.92	331.34	0.54

24.34	2,6-Dimethylquinoline	$C_{11}H_{11}N$	quinoline	158.96	157.21	2.70

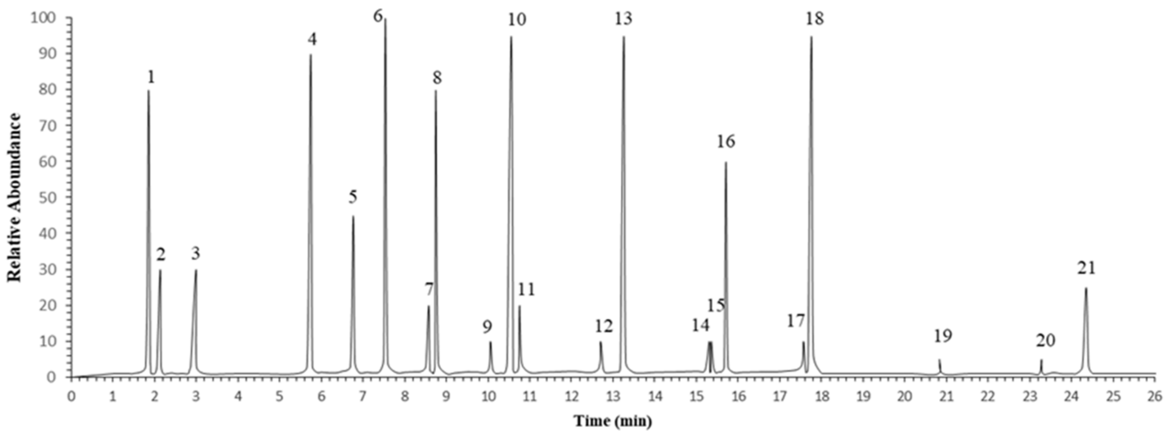
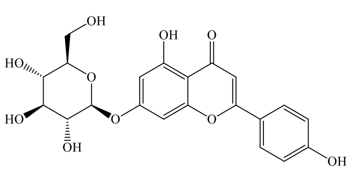


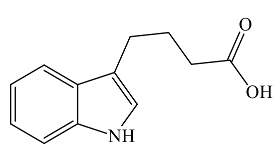
Figure 2.6 Chromatogram of compounds from ethanolic extract of bottle gourd leaves

2.3.6. Identification of biopesticide compounds from ethanol extract of bottle gourd leaf

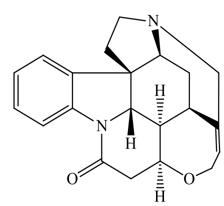
A literature search revealed that, among these constituents, apigenin-7-*O*-glucoside, indole-3-butyric acid, strychnine, phytol and hexadecanoic acid are highly effective in the management of cabbage looper (Figure 2.7).



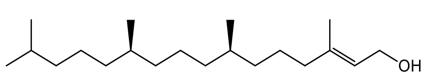
Apigenin-7-*O*-glucoside



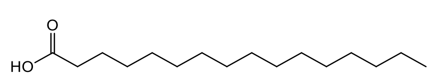
Indole-3-butyric acid



Strychnine



Phytol



Hexadecanoic acid

Figure 2.7 Structures of five biopesticide compounds identified by LC-MS in the bottle gourd leaf ethanol extract

2.3.7. FTIR spectra obtained from bottle gourd leaf extract (ethanolic)

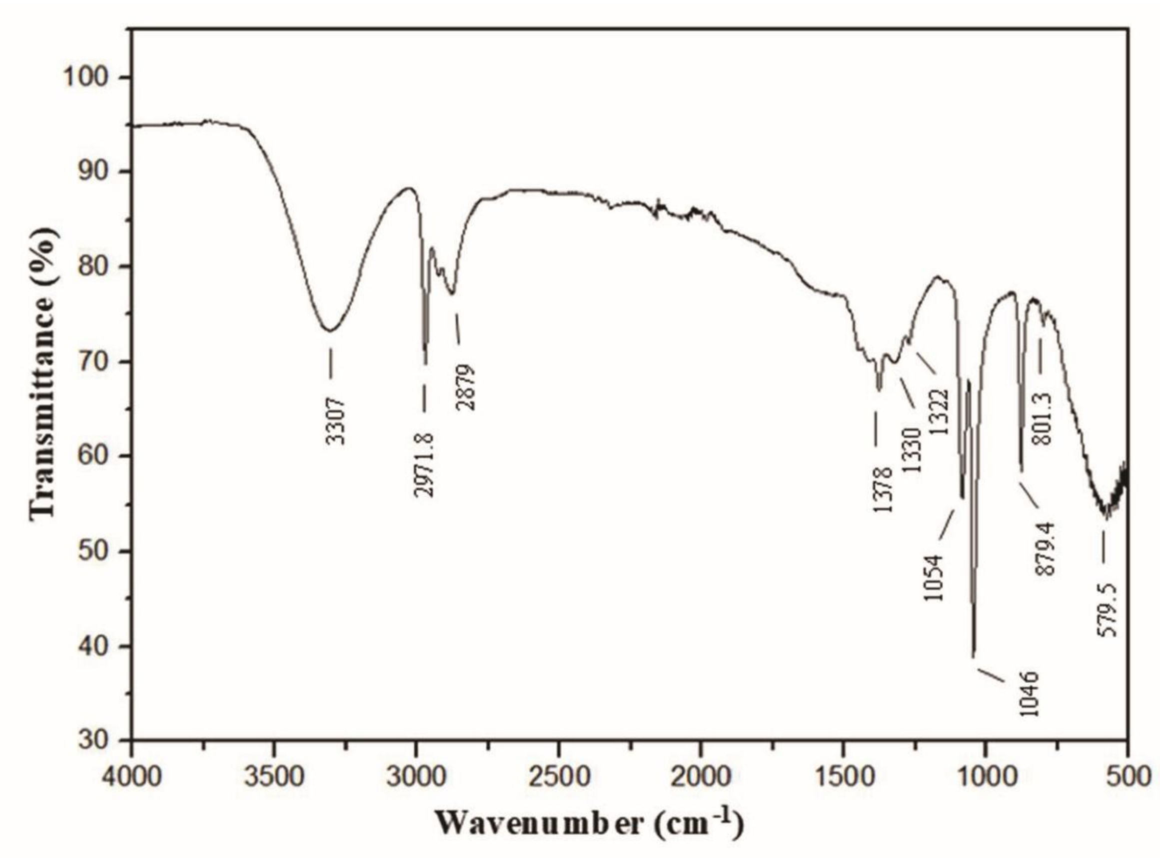


Figure 2.8 Fourier transform infrared spectroscopy spectrum of the ethanolic extract of bottle gourd leaves

FTIR analysis reveals the infrared spectrum of a material by collecting high-resolution spectral data over a specific spectral range and displays the extent of absorption of a single wavelength of infrared light by a sample at each wavelength (Nayak and Chakrabortty, 2023). The present FTIR analysis revealed absorption bands at 3307, 2971.8, 2879, 1378, 1330, 1322, 1054, 1046, 879.4, 801.3, and 579.5 cm^{-1}, indicating the presence of capping or stabilizing agents in the ethanolic extracts of bottle gourd leaves (Figure 2.8). The intense band at 3307 cm-1 corresponded to N–H stretching, which confirmed the presence of a primary aliphatic amine. The band at 2971.8 cm^{-1} corresponded to O-H stretching, confirming the presence of carboxylic acid. The peak at 2879 cm^{-1} corresponded to C–H stretching and confirmed the presence of alkanes. The peak at 1378 cm^{-1} was attributed to O-H bending and confirmed the presence of alcohol. The band observed at 1330 cm^{-1} was consistent with S=O stretching and indicated the presence of sulfone. The band at 1322 cm^{-1} corresponded to O-H bending and indicated the presence of phenol. The bands at 1054 and 1046 cm^{-1} were attributed to C-O stretching and CO-O-CO stretching, respectively, which indicated the presence of primary alcohol and anhydride, respectively. The bands at 879.4 and 801.3 cm^{-1} were attributed to C–H bending and C–C bending, respectively, which indicated the presence of a 1,2,4-disubstituted compound and an alkene, respectively. The 579.5 cm^{-1} band was consistent with C-Cl stretching and indicated the presence of a halo compound.

2.4. Discussion

Strong links have been established between exposure to pesticides and increased risk of several chronic diseases, such as various types of cancers, neurological disorders, cardiovascular diseases, developmental delays in children, effects on reproductive capacity, male and female infertility, cognitive impairments, and impaired respiratory health. Recent assessments have shown that pesticide exposure is linked to a broad range of direct and indirect effects on biodiversity, contributing to declines in populations of insects, birds, bats, earthworms, aquatic plants, fishes, and amphibians, among other organisms (Mamy, 2022).

Plant biopesticides are safe for both the applicant and the consumer because they are nontoxic (Damalas and Koutroubas, 2015). In addition, they do not affect beneficial organisms, such as natural enemies of pests, because they are target specific (Shiberu and Getu, 2016). Therefore, such substances can be effectively included in integrated pest management programs to decrease the volume of chemical pesticides used in the control of agricultural pests (Sesan *et al*., 2015). Furthermore, natural materials are safer to use in the environment because they decompose quickly (Kawalekar, 2013).

The plant chemical defense system comprises secondary metabolites, including phenols, flavonoids, quinones, terpenes, tannins, alkaloids, lectins, polypeptides, saponins, and sterols (War, 2012). Plant extracts rich in such secondary metabolites can disrupt cell membranes, inactivate a variety of enzymes, and disrupt the metabolic processes of insects, leading to growth inhibition or death. Plant extracts tend to have broad-spectrum activity, are relatively specific in their mode of action, and are easy to process and use at the farm level (Sadek, 2003).

The cabbage looper is a medium-sized moth in the family Noctuidae, a family that is frequently called "owlet moths." The larvae eat large holes in the underside of leaves and consume developing cabbage heads. Larvae initially do not consume much food but increase their consumption during their lifetime until they are consuming three times their weight daily (McEwen and Hervey, 1960).

Bottle gourd is an annual herbaceous plant with a prostrate growth habit (Harika, 2012). It is planted worldwide because of its high nutritional and medicinal properties (Attar, 2019). Ethnobotanical, epidemiological, and traditional information associated with the medicinal and nutritional capacity of bottle gourd has been thoroughly reviewed (Roopan *et al.*, 2016). A bottle gourd leaf extract shows larvicidal activity against third-instar larvae of *Musca domestica* (Hassan *et al*., 2018). A bottle gourd extract has been used as a natural insecticide against the mustard aphid *Lipaphis erysimi* (Mishra *et al.*, 2006). Like other members of the Cucurbitaceae, gourds contain cucurbitacins, which are known to be cytotoxic at high concentrations (Priya, 2010). Aldewy *et al*. tentatively identified 14 hexane-soluble compounds from air-dried leaves of bottle gourd by gas chromatography‒mass spectrometry (Aldewya *et al*., 2022).

In this study, the efficacy of a bottle gourd leaf extract was compared with those of the synthetic pesticide permethrin 3.2 EC and aqueous DMSO (the control). Compared with the control, treatment with the bottle gourd leaf extract dramatically reduced looper larval infestation in field-grown cabbage by 41.18%, 39.71%, 52.08%, and 37.96% at the fifth, sixth, seventh, and eighth weeks, respectively. The bottle gourd leaf extract exhibited strong biopesticide efficacy against cabbage looper, increased the cabbage yield by 15.98% compared with that of the control group, and approached the yield of cabbage plants treated with Permethrin 3.2 EC.

Twenty-one compounds were identified from the ethanolic extract of bottle gourd leaves by LC–MS analysis. Compared with other components, there are greater amounts of apigenin-7-*O*-glucoside

(2,2-dichloro-1-(4-methyl-benzoylamino)-vinyl)-phosphonic acid diethyl ester; 2-amino-9H-pyrido[2,3-b]indole; indolebutyric acid; phenol, 2-methoxy-4-(1-propenyl)-, acetate, (Z)-; phytol; 7-acetyl-1,3-dimethylpurine-2,6-dione; and (1Z)-1-[(3,4-diethoxyphenyl)methylidene]-6,7-diethoxy-3,4-dihydro-2H-isoquinoline and hexadecanoic acid. Among those compounds, apigenin-7-O-glucoside, a flavonoid, has been shown to have insecticidal effects on insects. It can also be used as a starting point for the development of new bioinsecticides. Apigenin-7-*O*-glucoside can kill insects and reduce their ability to lay eggs, making plants less appealing to insects, reducing their feeding and growth, and making it harder for insects to digest plants, thus reducing their survival (Mierziak *et al*., 2014; Hussein *et al*., 2022). Indole-3-butyric acid (IBA) has been used in combination with other chemicals to increase the resistance of plants to insects (D'Errico *et al.*, 2017). Strychnine is a pesticide used to control insects, rodents, and birds. It is also known as a rodenticide, an avicide, or an insecticide (Gupta., 2007). Phytol shows considerable potential for use in ecofriendly green insecticides (Benellia *et al*., 2020). Hexadecanoic acid has nematicide and pesticide activities (Sheela and Uthayakumari, 2013). The presence of primary aliphatic amines, carboxylic acids, alkanes, alcohols, sulfones, phenols, primary alcohols, anhydrides, 1,2,4-disubstituted, alkenes, and halo compounds was validated by the FTIR analysis presented in table S3. The present findings showed that the bottle gourd leaf extract functions as an effective insecticide against cabbage looper larvae. In future research, it will be useful to further improve the formulation of the bottle gourd leaf extract for practical applications to control cabbage looper crop infestation under field and laboratory conditions.

2.5. Conclusion

Compared with the control and permethrin 3.2 EC treatments, treatment with the bottle gourd leaf extract reduced cabbage looper infestation and improved cabbage productivity. Apigenin-7-*O*-glucoside, indole-3-butyric acid, strychnine, phytol and hexadecanoic acid are target compounds identified in the extract. Bottle gourd leaf extract can be used as an eco-friendly biopesticide instead of as a toxic chemical pesticide to control cabbage looper in a sustainable way. Because the current study was a small-scale field trial, additional research is needed to assess the potential practical use of bottle gourd leaves as biopesticides.

Chapter 3 Efficacy of black plum (*Syzygium cumini* L. Skeels) leaf extract against the 28-spotted potato ladybird (*Henosepilachna vigintioctomaculata*) on field-grown potatoes

3.1. Introduction

Botanical pesticides (biopesticides) derived from plants offer rapid biodegradation and low toxicity to non-target organisms and have long been promoted as appealing substitutes for synthetic chemical pesticides in the control of pests (Kanwal *et al*., 2021). Although synthetic pesticides are highly effective and fast-acting, they can pose significant environmental risks and health hazards, including harming beneficial insects and producing persistent residues. Furthermore, synthetic pesticides have high production costs and can negatively affect international trade because of residue limits. Consequently, biopesticides are favored in organic farming. When applied correctly, biopesticides have a much shorter preharvest withholding period than synthetic pesticides. This means they can be used on fresh produce to contribute to sustainable, climate-smart agricultural practices (Milton *et al*., 2025; Daraban *et al.,* 2023).

After rice, wheat, and maize, potatoes rank as the fourth most significant food crop worldwide (Çalışkan *et al*, 2023). Japan produces 2.4 million tons of potatoes per year, with 80% of this yield grown in Hokkaido prefecture (Tani *et al*., 2022). Potatoes are susceptible to a variety of diseases and pest insects that can greatly affect the yield and quality. Common diseases include late blight (*Phytophthora infestans*), early blight *(Alternaria solani*), common scab (*Streptomyces scabies*), and various viruses. Key pest insects are 28-spotted potato ladybirds (*Henosepilachna vigintioctomaculata*), Colorado potato beetles (*Leptinotarsa decemlineata*), aphids (*Myzus persicae*), potato tuber moths (*Phthorimaea operculella*), wireworms, and potato leafhoppers (*Empoasca fabae*). The 28-spotted potato ladybird is a common potato pest that is found in temperate areas of Asia, including China, Japan, Korea, and Russia. It belongs to the Coccinellidae family, whose members exhibit remarkable diversity, both in terms of their diets and the colored spots that emerge on the elytra in the adult stage. Potato leaves are consumed by both adults and larvae, which in extreme infestations results in the total defoliation and eventual death of the potato plant. Larvae often begin feeding on the undersides of infected leaves (Jackson 2017; Zhou *et al*., 2015). Eventually, the infected leaves get dry and wither. Additionally, gray mold infections (*Botrytis cinerea* Pers. ex Fr.) may be facilitated by feeding damage (Yao *et al*., 1992). Potato yield losses caused by this ladybird have been estimated to reach 10%–15% in normal years and 20%–30% in heavy infestation years (Song *et al*., 2008).

Potato ladybirds lay clusters of 15–50 eggs on lower leaf surfaces, with 150–750 eggs laid over a ladybird's lifetime. Hatching larvae disperse over short distances and start feeding. There are four to seven instars and with the molting of each instar there is an increase in antennal segments (Radcliffe and Lagnaoui, 2007). Pupation takes place on leaf surfaces. Development rates are dependent on temperature and the life cycle from a newly laid egg to a reproductive adult is concluded in 30–40 days under typical field conditions. The lifespan of an adult male is four months, and that of an adult female is three months. Females have a 45–80 day reproductive cycle (Huang *et al*., 2018; Wang *et al*., 2017). Consequently, the generations of beetles in the field significantly overlap with one another. Although adults are capable of flying, nothing is known about how they fly scientifically (Kovalenko and Pronyushkina, 2021).

Phytochemicals are naturally occurring chemicals present in or extracted from plants (Frank *et al*., 2020). In the plant defense system, compounds such as alkaloids, terpenoids, and phenolics that are toxic, act as repellents, or deter feeding by reducing the leaf nutritional content and increasing toughness are produced from plant phytochemicals (War *et al*., 2012; Adedayo *et al.*, 2024). Black plum (*Syzygium cumini* L. Skeels) in the family Myrtaceae is an evergreen tropical tree that is native to the Indian subcontinent and Southeast Asia (Morton, 1987). The essential oil of black plum is a promising candidate for antifungal and anti-*Trichomonas vaginalis* applications (Silva *et a.l*, 2024). This essential oil has antibacterial properties and is highly acidic in nature (Rizvi, 2022). Black plum leaf extract shows potential as an alternative to chemical fungicides used to control potato late blight and can be used to manage root-knot nematodes (Javed and Siddiq, 2021; Islam *et al*., 2021; Salim *et* *al.*, 2009). Compared with the control treatment, mancozeb treatment increased the yield by 36.16% and jamun treatment increased the yield by 30% (Bohora and Sapana, 2024). So, the black plum leaves extracts may have many biopesticide compounds which can control insect pests on potatoes.

A field experiment was conducted on potato plants to analyze and verify the effect of the extract on 28-spotted potato ladybirds. The phytochemical consequences of the extract on the potato development and production were evaluated, and the biopesticide compounds of the extract were determined.

3.2. Materials and methods

3.2.1. Preparation of the experimental site and plot

A field experiment was carried out in the late spring crop season from May 19, 2025, at the Field Science Center for Northern Biosphere, Hokkaido University (Sapporo, Japan). The experimental site was located at 43°4′ N latitude and 141°20′ E longitude, 20 m above sea level. Meteorological conditions from the seedling stage to harvest are listed in Table S1 and the soil properties during cultivation are detailed in Table S2. A randomized complete block design was conducted with three replicates of 10 plants for each treatment. The plot size per unit was 1.8 m × 1.5 m, the distance between the blocks (i.e., between replicates) was 0.75 m, the spacing between individual plants was 0.30 m, and the spacing between rows was 0.5 m. Five plowing operations, followed by laddering, were used to prepare the soil for planting. The field was leveled and cleared of uprooted weeds and crop stubble before seed potatoes were planted. Before transplanting the seed potatoes, 216 g of fertilizer (N:P:K = 8:20:14) was incorporated into the soil in every block.

The determinate potato variety used in this experiment, ‘Danshaku’, is popular with local growers and was purchased from the Hokuren Shop FoodFarm (Sapporo, Hokkaido, Japan). The field was irrigated eight times during the growing season.

3.2.2. Preparation of treatment and control solutions

Black plum leaves were imported from Rajshahi, Bangladesh. Visual inspection was performed to check for infections, spores, damage, discoloration, or distortion in the samples. After properly cleaning the undamaged leaf samples with running tap water, they were left to dry at room temperature in the presence of natural light. A high-speed grinder (WB-1, Osaka Chemical Co., Ltd., Osaka, Japan) was used to grind the dried leaves into a powder. A conical flask containing about 100 g of the powder was filled with 400 mL of ethanol. The conical flasks were shaken for 74 hours at 120 rpm in a Bioshaker BR-41FL, a constant-temperature incubator shaker manufactured by TAITEC Co. in Koshigaya City, Saitama, Japan. After centrifugation (Tomy MX-305 high-speed refrigerated microcentrifuge, Tomy Kogyo Co., Ltd, Fukushima, Japan) for 20 min at 2075 ×*g*, the extract was filtered using Whatman No. 1 filter paper. The ethanol was evaporated, and a crude extract was obtained by placing the filtrate in an EYELA-type constant-temperature air-drying oven (WFO-420W, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 50°C. Then, 1 g of the crude extract was diluted in 1 mL of dimethyl sulfoxide (DMSO) and 1000 mL of distilled water was added (Figure 3.1). The efficacy of the crude extract was compared with that of a control (0.1% v/v aqueous DMSO) and permethrin (3.2 EC formulation, 1% v/v aqueous solution). The dosage of permethrin was 1 mL per 1 L of water. Due to its widespread use, low toxicity to animals, and effectiveness against insects, permethrin was chosen for this study. The sodium channel current is disrupted by permethrin's action on the nerve cell membrane, delaying repolarization and paralyzing the insect. DMSO was chosen because it is an effective solvent for a variety of organic and inorganic substances and can be used to dilute plant extracts that might include a variety of different chemicals. Additionally, plants and animals can safely be exposed to DMSO concentrations below 0.1% (Gallardo-Villagrán *et al*., 2022).

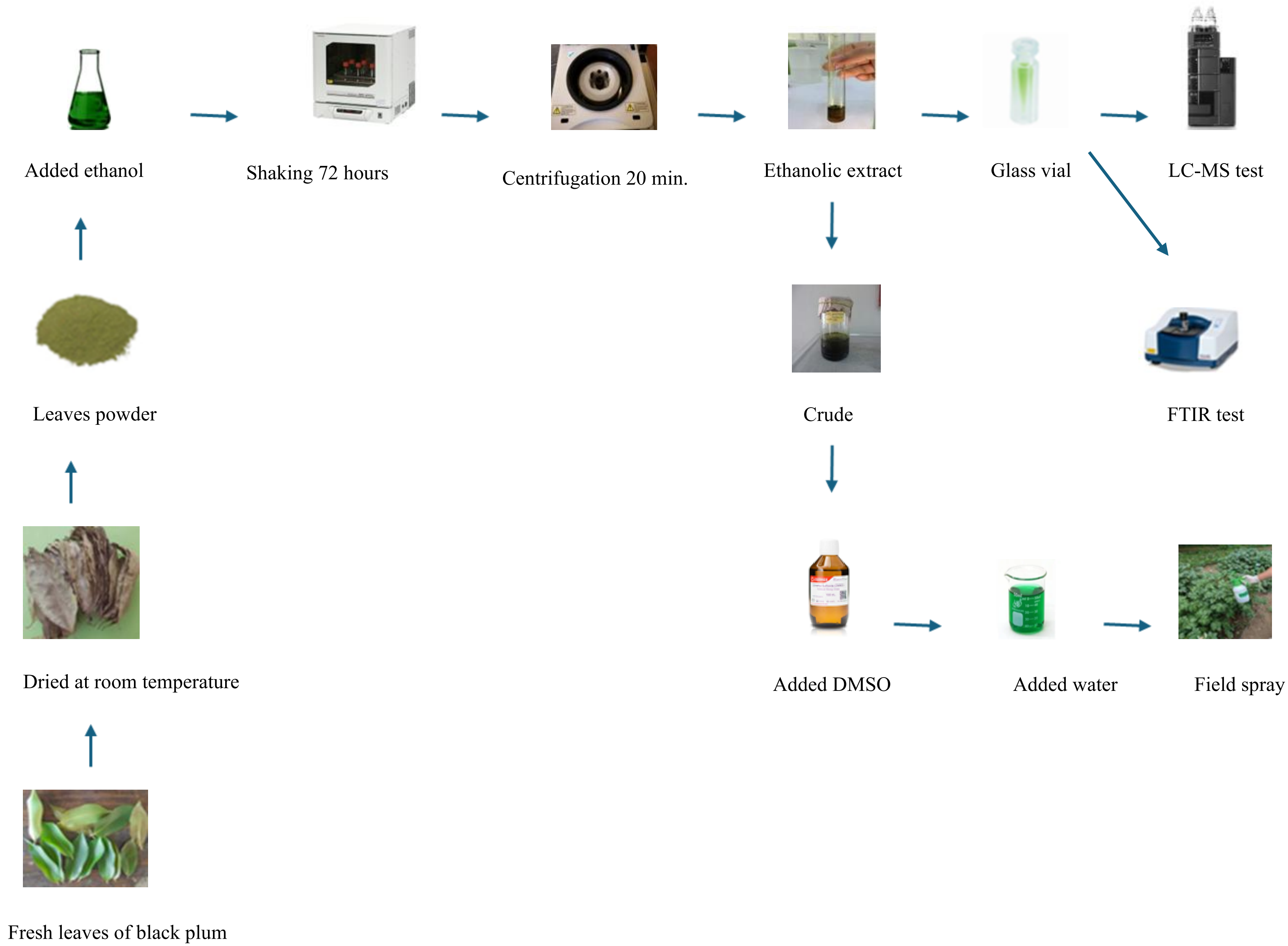


Figure 3.1 Procedure of black plum leaves extract preparation

3.2.3. Spray application of solutions

The prepared solutions (leaf extract, permethrin, and DMSO) were sprayed over the potato seedlings in the experimental field by using a 1.5-L hand-held garden sprayer (Taizhou City Hangyu Plastic Co., Ltd., Zhejiang, China) in the morning (09:00) at seven-day intervals (Figure 3.2 A). The spray treatments were initiated 30 days after sowing (DAS) of the seed potatoes and carried out until the growing season was over (73 DAS). Throughout this time, a total of seven applications of the treatment and control solutions were made to potato plants, using 10 mL per plant.

Figure 3.2 Photographs of the plants during the experiment: (A) spraying, (B) infected potato plants and (C and D) 28-spotted potato ladybirds

3.2.4. Monitoring of 28-spotted potato ladybird infestation and plant growth indicators

During the present experiment, only the 28-spotted potato ladybird was visible on the plants from the seedling stage to the final growth stage. First, the number of 28-spotted potato ladybirds on each infected plant was counted (Figure 3.2 B). Infestation of 28-spotted potato ladybirds on the potato plants was monitored from the start of insect colonization to potato plant maturity (Figure 3.2 C and Figure 3.2 D). Other pests were not taken into account because they were present in minuscule amounts. The plots were sampled every day by visually inspecting both leaf surfaces in the morning from July 7 to 30. Each plot had ten potato plants, and the number of 28-spotted potato ladybirds within the canopy was tallied.

Plant growth indicators were measured in the field in August 2025. Yield data were collected after harvest on August 6 (80 DAS) (Figure 3.3). The plant height by centimeter scale and stem diameter by using slide calipers (in centimeters) were measured, and then the leaf chlorophyll content was estimated using a portable chlorophyll meter (SPAD-502, Minolta Camera Co., Ltd., Osaka, Japan) (Figure S1A). A plant photosynthesis metre (miniPPM-300, EARS, Delft, the Netherlands) was used to assess the rate of photosynthesis (Figure S1B).

The total potato yield (kg/plot) was noted and the percentage yield compared with that of the control was calculated using Eq. 1.

$$\text{Yield (\%)} = \frac{\text{treatment yield} - \text{control yield}}{\text{control yield}} \times 100. \qquad \text{(Eq. 1)}$$

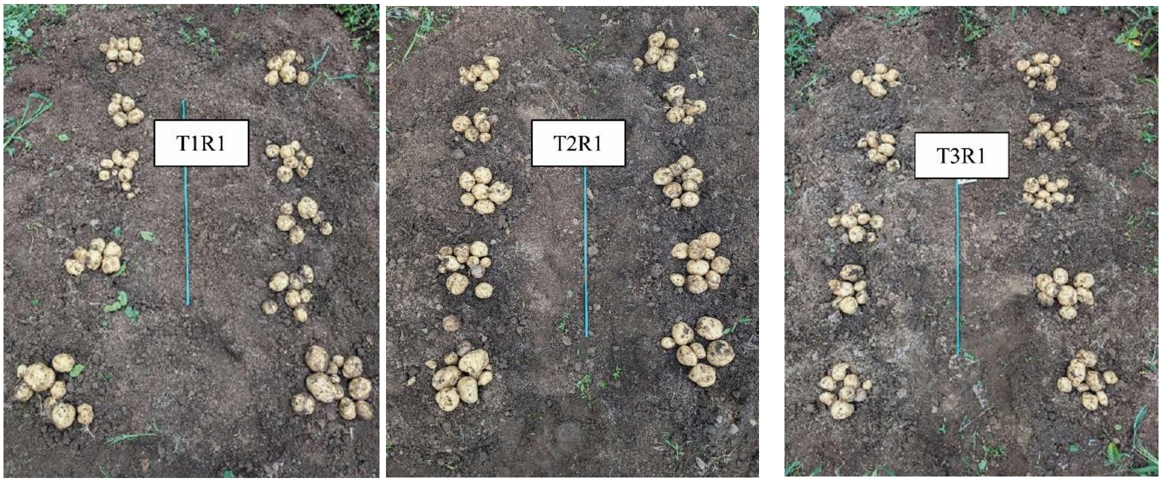


Figure 3.3 Potato production each plot of treatment

The rate of 28-spotted potato ladybird infestation (%) compared with that in the control group (treated with aqueous DMSO) was calculated using Eq. 2.

$$\text{Infestation rate (\%)} = \frac{\text{beetles in treatment group} - \text{beetles in control group}}{\text{beetles in control group}} \times 100. \qquad \text{(Eq. 2)}$$

3.2.5. Sample preparation for phytochemical analysis

The black plum leaf ethanol extract was prepared for analysis following standard procedures. All of the reagents and solvents utilized for liquid chromatography-mass spectrometry (LC–MS) were of high-performance liquid chromatography grade (Rajapaksha, 2022).

3.2.5.1. LC–MS analysis

The black plum leaf ethanol extract was subjected to LC–MS analysis using a mass spectrometer (LTQ-Orbitrap XL, Thermo Fisher Scientific, Waltham, MA, USA) connected to a liquid chromatograph (Nexera-XR, Shimadzu, Kyoto, Japan). The extract (5 µL) was injected into an Inert Sustain AQ-C18 column (100 × 2.1 mm, 1.9 µm; GL Sciences, Tokyo, Japan) maintained at 40°C. The mobile phase was a combination of aqueous formic acid (0.1%, solvent A) and 0.1% formic acid in acetonitrile (solvent B). Gradient elution was carried out at 150 µL/min as follows: 0 minutes at 50% B, 15 minutes at 95% B, 20 minutes at 95% B, 21 minutes at 50% B, and 26 minutes at 50% B.

For mass spectrometry, ionization by electrospray was employed in positive mode. The other instrument settings were as follows: source voltage, 4.0 kV; nitrogen sheath gas flow rate, 30 L/ minutes; auxiliary gas flow rate, 10 L/ minutes; flow rate of sweep gas, 0 L/ minutes; capillary temperature, 300°C; capillary voltage, 20 V; and tube lens voltage, 80 V. All extracts were evaluated in Fourier transform mass spectrometry mode with full-range mass spectra acquired over *m*/*z* 100–2000. Usually, solvent A was used to dilute the samples (1:200). XCalibur software v. 2.0.7 (Thermo Fisher Scientific) was used to analyze the data.

3.2.5.2. Fourier transform infrared spectroscopy

The functional groups and molecular structures in the black plum leaf ethanol extract were investigated using Fourier transform infrared spectroscopy (FTIR; FT/IR-4X, JASCO Corporation, Tokyo, Japan). For each spectrum, 50 scans with a resolution of 4 cm−1 that were recorded between 500 and 4000 cm−1 were averaged. To guarantee accurate measurements, the baseline was modified for atmospheric CO2 and water vapor.

Additionally, hexane extract of black plum leaf was made by using the same procedure only for laboratory test.

3.2.6. Statistical analysis

The biopesticide activity of the black plum leaf extract against 28-spotted potato ladybirds on field-grown potato plants was evaluated in an experimental plot using a randomized complete block design. The data were entered into Excel (Microsoft, Redmond, WA, USA) and subjected to one-way analysis of variance. Significant differences across treatments were revealed using Duncan's multiple range test ($P < 0.05$) using IBM SPSS Statistics v. 20 software (IBM Corp., Armonk, NY, USA).

3.3. Results

3.3.1. Effects of black plum leaf ethanol extract on 28-spotted potato ladybird infestation on potato plants

The chemical insecticide permethrin provided the best defense against 28-spotted potato ladybirds. However, compared with the control (aqueous DMSO), the black plum leaf ethanol extract had good efficacy against the ladybirds and significantly decreased 28-spotted potato ladybird larval infestation in field-grown potatoes by 71.43%, 52.94%, 47.62%, and 63.33% at 50 DAS, 58 DAS, 66 DAS, and 73 DAS, respectively. By comparison, with application of aqueous DMSO, the infestation rate increased daily to reach 100% at harvest. With the black plum leaf extract, good protection was provided against 28-spotted potato ladybirds and the infestation rate increased slowly between 50 to 66 DAS and then did not increase after 66 DAS (Figure 3.4).

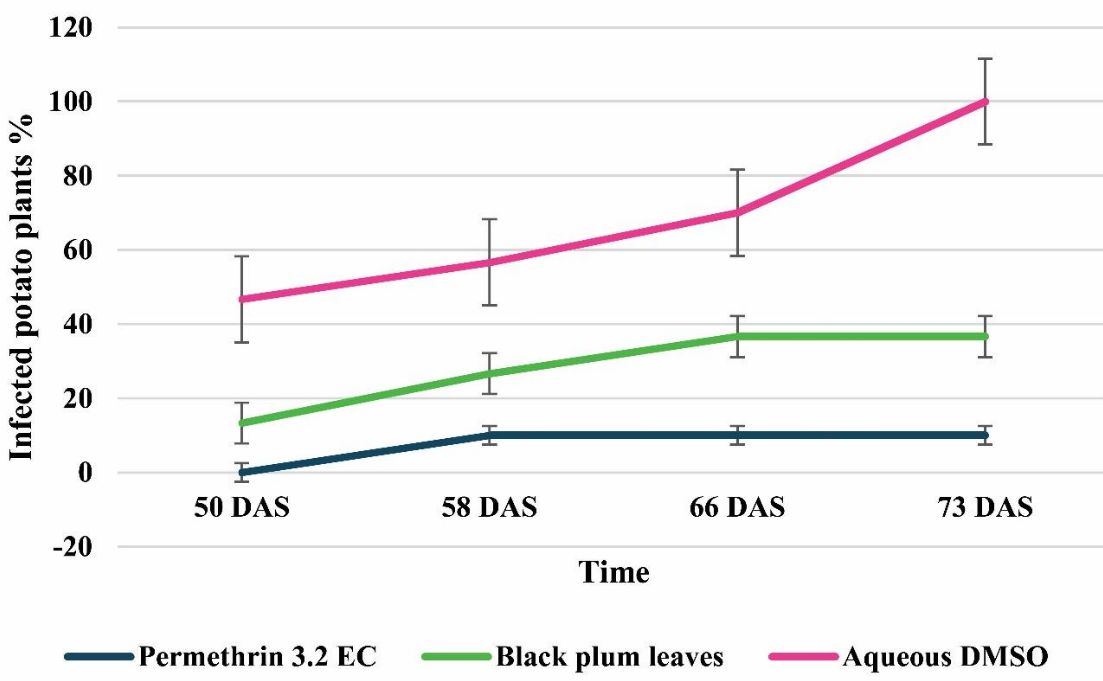


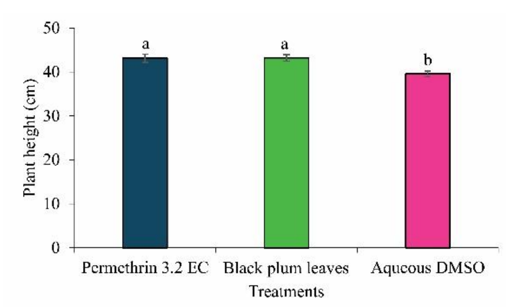
Figure 3.4 Activity against 28-spotted potato ladybird infestation on potato plants for the black plum leaf extract, permethrin, and aqueous DMSO. The error bars represent the standard error ($P < 0.05$, $n = 30$)

3.3.2. Effect of black plum leaf ethanol extract on the relative chlorophyll content, photosynthesis rate, and plant growth indicators

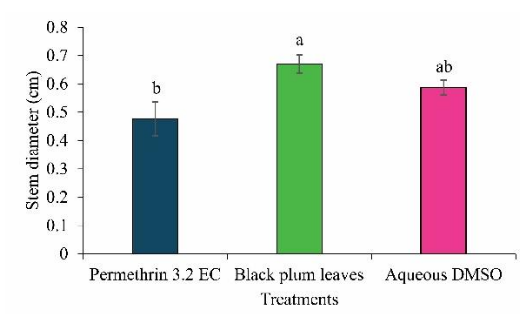
No significant differences were found in the plant height among the treatment and control groups, but in stem diameter there was significant difference found because the black plum leaf extract group showed best result than other groups (Figure. 3.5 A and 3.5 B). No significant differences in the relative chlorophyll contents or photosynthesis rates were found among the treatment and control groups (Figure. 3.5 C and 3.5 D).

3.3.3. Effects of the black plum leaf ethanol extract on potato yields and plant biomass

The permethrin and black plum leaf extract treatments produced potato yields of 28.17 and 31.46 mt/ha, respectively, which showed that protection against 28-spotted potato ladybirds resulted in good yields. By contrast, the potato yield with the control (aqueous DMSO) was lower at 27.58 mt/ha, which was related to the low efficacy of DMSO against 28-spotted potato ladybird infestation (Section 3.1). The black plum leaf extract increased the potato yield by 14.06% compared with aqueous DMSO and 11.68% compared with permethrin (Figure 3.5 E). Black plum leaf extract treatment also resulted in higher plant biomass (30.71 mt/ha) than permethrin (28.74 mt/ha) or aqueous DMSO (27.83 mt/ha) (Figure 3.5 F).



A



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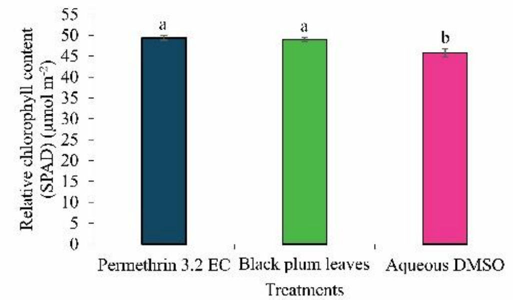

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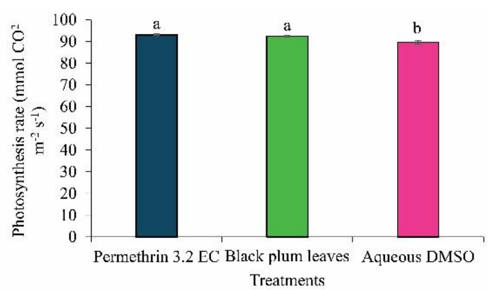

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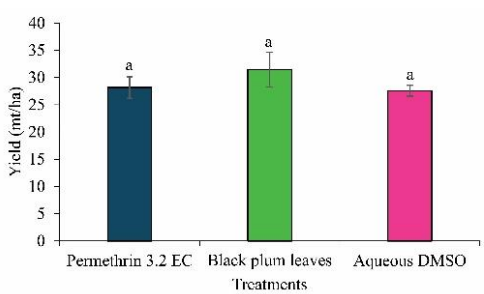

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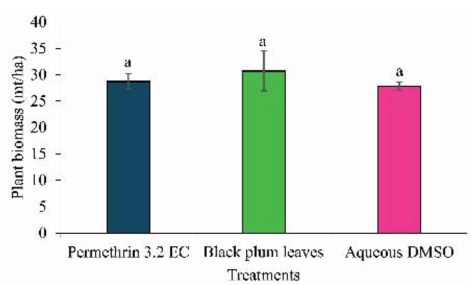


F

Figure 3.5 Effects of the black plum leaf ethanol extract on (A) plant height, (B) stem diameter, (C) relative chlorophyll content, (D) photosynthesis rate, (E) potato yield, and (F) plant biomass. Different lowercase letters above bars point to a significant difference as determined by Duncan's multiple range test and error bars represent the standard error (*P* < 0.05; *n* = 30)

3.3.4. Characterization and identification of biopesticide compounds in the black plum leaf ethanol extract by LC–MS

LC–MS examination of the black plum leaf ethanol extract enabled identification of 16 chemicals (Table 3.1 and Figure 3.6).

Table 3.1 Tentatively identified compounds in the black plum leaf ethanol extract and their retention times, chemical names, elemental formulae, detected *m/z*, and exact *m/z*.

R/T	Detected M/Z	Exact m/z	Formula	Chemical name	% of Conc.	Class
9.15	271.19	270.05	$C_{15}H_{10}O_5$	3,7,4'-Trihydroxyflavone	2.3283	flavonoid
10.52	273.17	272.07	$C_{15}H_{12}O_5$	3-(5-Acetyl-2-furyl)-5-methoxy-2-benzofuran-1(3H)-one	3.7252	furanolactone
10.53	397.38	396.30	$C_{27}H_{40}O_2$	Delta-Tocotrienol	5.5879	terpenoid
11.19	196.16	195.22	$C_{10}H_{13}NO_3$	(3S)-3-amino-4-(4-hydroxyphenyl)butanoic acid	0.1164	beta-amino acid
11.50	635.29	634.41	$C_{36}H_{58}O_9$	3-GluA Soyasapogenol B	11.6414	saponin
11.60	593.27	592.55	$C_{28}H_{32}O_{14}$	Acacetin-7-*O*-rutinoside	11.6414	flavonoid
12.36	411.25	410.14	$C_{23}H_{22}O_7$	Amorphigenin	11.6414	rotenoid
12.90	621.31	620.56	$C_{29}H_{32}O_{15}$	Flavone C-glycosides	3.4924	flavonoid
13.00	425.27	424.67	$C_{29}H_{44}O_2$	alpha-Tocotrienol	11.6414	terpenoid
13.22	436.34	435.54	$C_{18}H_{33}N_3O_7S$	Glutamyl-S-($C_8H_{17}O$)-cysteinylglycine	0.9321	oligopeptide
13.83	441.30	440.13	$C_{19}H_{19}N_7O_6$	Folic acid	11.6414	pterin
13.94	338.34	337.34	$C_{22}H_{43}NO$	Erucamide	0.5821	fatty acid
15.73	902.52	900.23	$C_{42}H_{44}O_{22}$	Genistein -7-*O*-Glc-Xyl, Acetate	0.5821	isoflavone

16.25	663.45	662.82	$C_{36}H_{54}O_{11}$	Medicagenic acid base - H2O + *O*-HexA	11.6414	saponin
16.82	885.55	884.07	$C_{45}H_{73}NO_{16}$	Solasodine base + *O*-Hex-Hex-dHex	2.3283	alkaloid
17.55	871.57	870.54	$C_{50}H_{79}O_{10}P$	Phosphatidylglyceride 22:6-22:4	10.4773	phospholipid

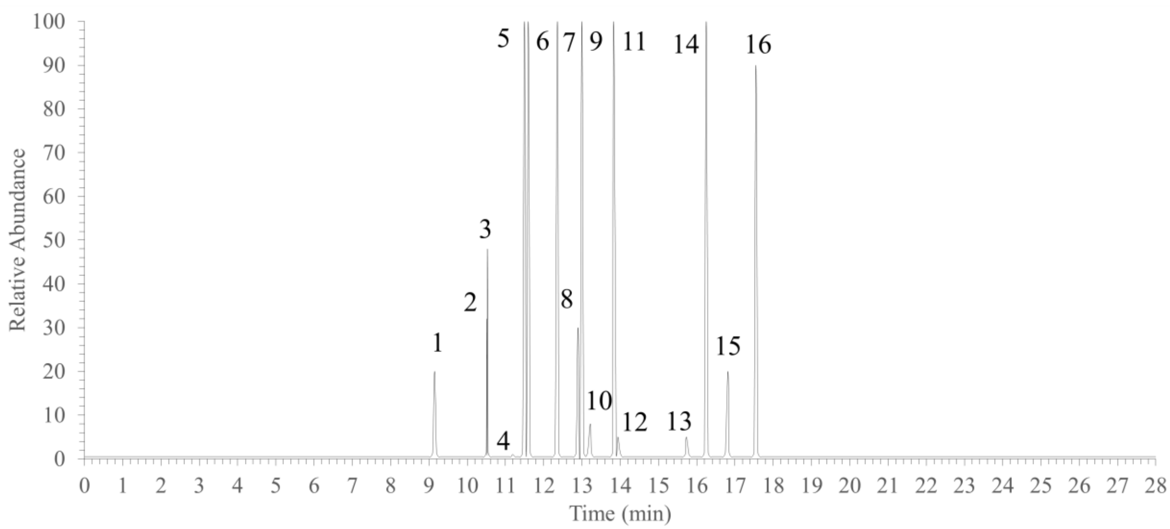
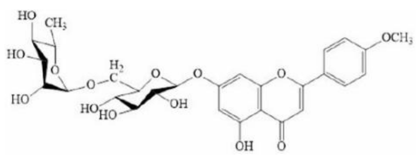


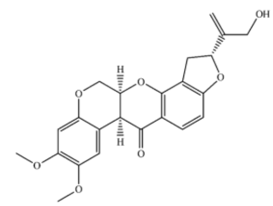
Figure 3.6 Chromatogram of compounds from ethanolic extract of black plum leaves

3.3.5. Identification of biopesticide compounds from ethanol extract of black plum leaf

Among the sixteen compounds detected in the black plum leaf extract, two were identified as biopesticides by comparison with the literature search (Figure 3.7).



and



Acacetin-7-*O*-rutinoside **Amorphigenin**

Figure 3.7 Structures of two biopesticide compounds identified by LC-MS in the black plum leaf ethanol extract.

In this study, a black plum leaf hexane extract was also analyzed by LC–MS (Table S4).

3.3.6. FTIR analysis of the black plum leaf ethanol extract

FTIR analysis of the black plum leaf ethanol extract revealed peaks at 577.6, 879.4, 1047.2, 1114.7, 1263.2, 1376, 1457, 2162.8, 2880.2, 2926.5, 2972.7, and 3298.6 cm^{-1}, the extract (Figure 3.8). The peaks at 3298.6 cm^{-1} and 2162.8 cm^{-1} were attributed to C–H stretching and C≡C stretching of alkynes. The peak at 2972.7 cm^{-1} corresponded to N–H stretching (amine salts). The peaks at 2926.5 and 2880.2 cm^{-1} corresponded to O–H stretching for alcohol and carboxylic acid groups, respectively. The peak at 1457 cm^{-1} corresponded to C–H bending (alkanes). The intense peak at 1376 cm^{-1} corresponded to O–H bending (phenols). Peaks at 1114.7 and 1263.2 cm^{-1} were attributed to C-O stretching for aromatic ester groups and aliphatic ester groups, respectively. The peak at 1047.2 cm^{-1} was attributed to CO–O–CO stretching (anhydrides). The peak at 879.4 cm^{-1} was attributed to C=H bending and stretching (1,3-disubstituted compounds), and the 577.6 cm^{-1} band was attributed to C–Cl stretching (halogenated compounds) (Table S5). A black plum leaf hexane extract was also subjected to FTIR analysis in this investigation (Table S6).

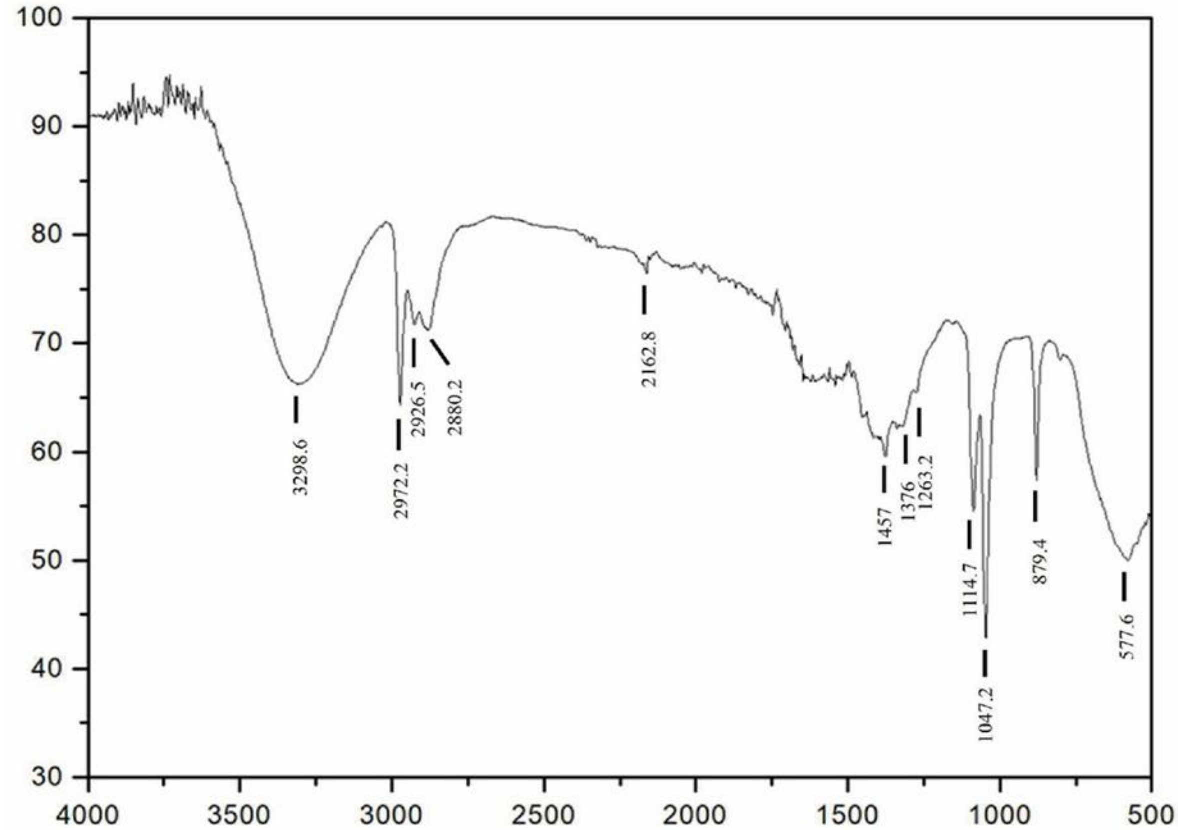


Figure 3.8 Fourier transform infrared spectrum of the black plum leaf ethanol extract

3.4 Discussion

Biopesticides are pesticides derived from natural sources such as plants, animals, bacteria, or certain minerals that provide an alternative to synthetic chemicals for controlling pests. Biopesticides are considered eco-friendly and host specific and contribute to sustainable agriculture by minimizing residues in food and reducing pesticide resistance compared with traditional chemical pesticides (Mawcha *et al.*, 2025).

The 28-spotted potato ladybird beetle is a large, herbivorous beetle that has dull orange wing cases with black spots and a surface covered in short hairs. It is a major pest of potatoes and cucurbits, and feeds on leaves, which results in them drying up and dying. During the beetle life cycle, oval, yellow eggs are laid in batches on leaf undersides, and pale yellow, spined larvae hatch that also graze on leaves.

Adults are approximately 10-mm long, and both adults and larvae are covered in short hairs (Ermak and Matsishina, 2022). Spraying of aqueous neem seed extract at intervals can reduce 28-spotted potato ladybird populations (NRCPN, 1992). Tobacco leaf extracts with concentrations of 5% and 10% can also be used to control the 28-spotted potato ladybird, especially in integrated management strategies (Weber *et al*., 2019). A 5% Karanja (*Millettia pinnata*) plant extract has also demonstrated effectiveness in managing these beetles (Ghosh and Chakraborty, 2012). In this study, black plum leaf extract was used as a biopesticide against 28-spotted potato ladybirds in an experimental potato field.

In LC-MS analysis of the black plum leaf extract, the dominant components were 3-GluA soyasapogenol B, acacetin-7-*O*-rutinoside, amorphigenin, α-tocotrienol, folic acid, medicagenic acid - H_2O + *O*-HexA, and phosphatidylglyceride 22:6-22:4 (Figure S2). The nervous system of the 28-spotted potato ladybirds can be disrupted by synthetic pyrethroids or through toxins ingested from their prey (Schmidtberg *et al*., 2019). From literature survey it is found that acacetin-7-*O*-rutinoside is harmful to true bugs (insects of the order Hemiptera) and true flies (insects of the order Diptera) but is harmless to honeybees. In plants, this compound functions in defense by deterring some plant-feeding insects and could play a role in regulating plant growth and development (Riddick, 2024). Amorphigenin is a rotenoid compound found in plants like *Amorpha fruticosa* that acts as an insecticide by inhibiting mitochondrial complex I (NADH:ubiquinone oxidoreductase) in insects, which disrupts cellular respiration and leads to death. This compound can interfere with plant development by suppressing seed germination and seedling growth (Liang *et al*., 2015; Ji *et al*., 2015; Kozuharova *et al*., 2025). Flavone C-glycosides are potential biopesticides that have a broad target spectrum, including insects, fungi, and other organisms. In plants, these compounds function as antioxidants, UV absorbers, and flower pigments, and play roles in plant defense against insects and microbes (Pereira *et al*., 2024). Erucamide has potential for use in eco-friendly pesticides. This compound functions in plant defense against insects by directly disarming them rather than killing them (Bao *et al*., 2025, Ikram *et al*., 2025).

Fifteen components were detected by LC–MS in the black plum leaf hexane extract (Figure S2). These compounds were lupanine, aspidocarpine, acetylated camptothecin, lysophosphatidylcholine 18:2, *N*-fructosyl glutamylphenylalanine, pyrrhoxanthinol, daidzein-8-C-glucoside, medicagenic acid + *O*-Hex, catechin gallate, isomajdine, gardneramine, solanidine + *O*-Hex-Hex, ergocryptine, 2,3,4-trihydroxybutanal, and bayogenin + *O*-HexA. Among these compounds, four are biopesticides (Figure S3). These compounds are lupanine, acetylated camptothecin, daidzein-8-C-glucoside, and catechin gallate. Lupanine exhibits insecticidal and antifeedant properties, making it a potential natural pesticide (Cely-Veloza *et al*., 2022; Boschin and Resta, 2013). Acetylated camptothecin shows promise as an insecticide and pesticide because of its ability to induce cell death and inhibit insect growth and is a potential biopesticide (Yang *et al*., 2019; Sloan *et al*., 2017; Zhang *et al*, 2012). Daidzein-8-*C*-glucoside has insecticidal properties and can inhibit the growth and development of some insect larvae (Punia and Chauhan, 2022; Murakami *et al.,* 2014; Qiang *et al*., 2022). Catechin gallate has demonstrated insecticidal effects in studies involving aphids (*Aphis gossypii*) and the pod borer (*Helicoverpa armigera*) (Zhao *et al*., 2021).

In this study, a black plum leaf hexane extract was also analyzed by FTIR. FTIR analysis revealed peaks at 584.3 cm^{-1} for halogenated compounds, 824.4 cm^{-1} for alkenes, 867 cm^{-1} for 1,3-disubstituted compounds, 972.9 cm^{-1} for alkenes, 1064.5 cm^{-1} for primary alcohols, 1378 cm^{-1} for phenols, 1457 cm^{-1} for alkanes, 1602 cm^{-1} for conjugated alkenes, 2283.3 cm^{-1} for carbon dioxide, 2858 cm^{-1} for alkanes, 2922.6 cm^{-1} for alcohols, and 2957.3 cm^{-1} for amine salts in the black plum leaf hexane extract (Figure S4).

Our findings show that the black plum leaf extract functions as an effective pesticide against 28-spotted potato ladybirds. In future studies, it will be beneficial to further development the formulation of the black plum leaf extract for practical application to control 28-spotted potato ladybird infestations under field and laboratory conditions. Acacetin-7-*O*-rutinoside and amorphigenin are the target compounds of this study because of their higher percentage of concentrations than other compounds.

3.5. Conclusions

This research reveals the insecticidal effects of black plum leaf ethanol extract against the 28-spotted potato ladybird on field-grown potatoes. Compared with aqueous DMSO and permethrin, black plum leaf extract reduced 28-spotted potato ladybird infestation and improved potato yields. The insecticidal effect may be attributed to the two main constituents, specifically the acacetin-7-*O*-rutinoside and amorphigenin identified by liquid chromatography–mass spectrometry analysis. Our results show black plum leaf extract can be used as an eco-friendly biopesticide instead of a toxic synthetic pesticide for sustainable control of 28-spotted potato ladybirds. The present study was a small-scale field trial; thus, more investigation is required to evaluate the potential usefulness of black plum leaf extract as a biopesticide.

Chapter 4 Conclusion and recommendation

4.1. Conclusion

Studies indicate biopesticides are eco-friendly, low-toxicity, biodegradable, and specific in action with little or no negative impact on non-target organisms. They work through various mechanisms, such as being parasitic or repelling pests, and have a diverse mode of action that can make them less prone to causing pest resistance compared to conventional chemical pesticides. Biopesticides are safe for human health as well as the environment, farmers can make them easily at home, cheaper than chemical pesticides. Modern agricultural food production provides high-quality crop products on an extremely large scale. This is achieved in part by the judicious use of pesticides that help maintain high yields by protecting crops from insects and diseases. To address these agricultural challenges, pesticides have become a fundamental technological advancement for enabling large-scale food production. The main focus was on development of biopesticide from bottle gourd (*Lagenaria siceraria*) leaves and black plum (*Syzygium cumini*) leaves for eco-friendly pest control in cabbage and potato fields. Because bottle gourd leaves and black plum leaves may have many bioactive compounds which can control cabbage looper and 28-spotted potato ladybirds respectively, reduce pollution and protect the human health and environment. This study determines the efficacy of bottle gourd leaf and black plum leaf extract in controlling cabbage looper and 28-spotted lady bird on field-grown cabbage and potato, effect of the extracts on the growth and yield of cabbage and potato, and finally analyze the biopesticide compounds of the bottle gourd leaf and black plum leaf, respectively. Summarizing the research based on the above content, the experimental results are as follows:

(1) In this study efficacy test of bottle gourd leaf extract against cabbage looper is done by small scale field trial compared with chemical pesticide and control aqueous DMSO. From this research it is found that compared with the application of permethrin, application of bottle gourd leaf extract significantly decreased the looper infestation by up to 41.18%, 39.71%, 52.08%, and 37.96% at the fifth, sixth, seventh, and eighth weeks, respectively. Bottle gourd leaf extract treatment not

only controlled looper but also increased the yield of cabbage compared with that of the control groups. Five possible biopesticide compounds, namely, apigenin-7-*O*-glucoside, indole-3-butyric acid, strychnine, phytol and hexadecanoic acid, were identified in the ethanolic extracts of bottle gourd leaves by liquid chromatography–mass spectrometry analysis. FTIR test confirmed the presence of the biopesticide components of bottle gourd leaf extract. This work lays a foundation for the agricultural application of bottle gourd leaves as a biopesticide and for improving cabbage cultivation.

(2) This research focused on how black plum leaf extract affects and reduces 28-spotted lady birds on potato fields and improves the production of potatoes. Application of the extract decreased 28-spotted potato ladybird infestation in field-grown potatoes by 71.43%, 52.94%, 47.62%, and 63.33% at 50 days after sowing (DAS), 58 DAS, 66 DAS, and 73 DAS, respectively, compared with the control. Besides insect control black plum leaf extract is also enhanced the potato yield by 14.06% compared with the control and 11.68% compared with permethrin. Two possible chemicals for biopesticides, acacetin-7-*O*-rutinoside and amorphigenin were identified by liquid chromatography–mass spectrometry analysis of the black plum leaf ethanol extract. This study not only demonstrates the application of environmentally friendly and sustainable alternative to toxic chemical pesticides for controlling 28-spotted ladybird infestation in potato field but also facilitates the characterization on black plum leaf extract.

4.2. Recommendation

This study has provided valuable insights into the biopesticide investigated. However, there are several avenues for further research that can contribute to a deeper understanding and advancement of the field. The following research recommendation highlights potential areas for further investigations:

(1) Production of crops and their insect pests vary from country to country and their different environment. In addition to plant extract, a variety of other variables including plant health, soil texture, water content, weather, fertilizer dosage, and intercultural operations etc. might affect

yield. In further research, it will be useful to further improve the formulation of the bottle gourd leaf extract for practical applications to control cabbage looper on crops under larger field conditions. LD50 is needed to determine the toxicity of the bottle gourd leaf extract, providing a standard measurement to compare different chemicals and assess potential hazards. Additionally, the quantity of bioactive compounds extracted in this study was limited, and analyzed by only LC-MS. Hence, further research on purification processes and GC-MS are necessary. This would not only complement the current study but also promote the industrial application of biopesticide industry.

(2) Potatoes can be affected by several diseases and insect pests which will be dependent on weather conditions and may vary place to place. Though it is a small-scale field trial for 28-spotted lady birds, more research on a large scale is needed for final confirmation. Besides black plum leaf purification, separation and bioassay with single compound is also needed for target biopesticide component. For more appropriate results and phytochemical toxicity of black plum leaf extract dose-response curves to determine LC50 values should be investigated on different insects in laboratory conditions. Though the potential of the identified components as biopesticide was found by literature survey, it may be further improved by laboratory experiment and observed their mode of actions on insects. GC-MS and NMR Spectroscopy are also helped to reach the black plum leaf as an ecofriendly biopesticide at an industrial level.

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Supplementary

Table S1 Climatic conditions of experimental sites during production season

	Air temperature (°C)		Precipitation (mm)
	Max	Min	Sum
April	11.6	1.667	0.10
May	21.67	11.11	0.10
June	30.6	8.8	50.5
July	34.3	16.4	7.5
August	34.4	13.9	108.5

Table S2 Characteristics of soil properties present in cultivation condition

Parameter	Unit	Weight
Attributes		Sandy soil
P-absorption		480
CEC	cmol kg^{-1}	0.97
N	g kg^{-1}	0.89
Olsen-P	mg 00 g^{-1}	6.4
K exchangeable	mg 100 g^{-1}	22
Ca exchangeable	mg 100 g^{-1}	157.8
Mg exchangeable	mg 100 g^{-1}	15.8
Cu	ppm	3.93
Zn	ppm	3.17
Mn	ppm	19.18
B	ppm	0.30

CEC cation exchange capacity

Table S3 Fourier transform infrared spectroscopy peak values and functional groups for the ethanolic extract of bottle gourd leaves

Sl. no.	Wave number (cm-1)	Intensity	Bond responsible	Functional groups
1	3307	strong, broad	N-H stretching	aliphatic primary amine
2	2971.8	strong, sharp	O-H stretching	carboxylic acid
3	2879	medium	C-H stretching	alkane
4	1378	strong	O-H bending	alcohol
5	1330	strong	S=O stretching	sulfone
6	1322	medium	O-H bending	phenol
7	1054	strong	C-O stretching	primary alcohol
8	1046	strong	CO-O-CO stretching	anhydride
9	879.4	strong	C-H bending	1,2,4-disubstituted
10	801.3	medium	C=C bending	alkene
11	579.5	medium	C-Cl stretching	halo compound

Table S4 Tentatively identified compounds in the hexane extract of Black plum leaves and the respective retention time (R/T), chemical name, elemental formula, detected m/z, and exact m/z.

R/T	Detected M/Z	Exact m/z	Formula	Chemical name	% of conc.	Class
4.02	249.21	248.19	$C_{15}H_{24}N_2O$	Lupanine	4.2056	alkaloid
6.70	371.32	370.23	$C_{22}H_{30}N_2O_3$	Aspidocarpine	0.7009	alkaloid
7.60	409.39	408.41	$C_{22}H_{20}N_2O_6$	Acetylated camptothecin	5.1402	alkaloid
10.05	520.43	519.66	$C_{26}H_{50}NO_7P$	LPC 18:2	0.4673	phospholipid
12.10	457.30	456.45	$C_{20}H_{28}N_2O_{10}$	N-Fructosyl glutamylphenylalanine	23.3644	peptide
12.36	572.47	570.34	$C_{37}H_{46}O_5$	Pyrrhoxanthinol	0.4673	terpenoid
12.99	417.30	416.38	$C_{21}H_{20}O_9$	Daidzein-8-*C*-glucoside	23.3644	flavonoid
14.00	665.62	664.83	$C_{36}H_{56}O_{11}$	Medicagenic acid base + *O*-Hex	1.1682	saponin
14.55	443.32	442.09	$C_{22}H_{18}O_{10}$	Catechin gallate	23.3644	Flavonoids
14.75	429.37	428.49	$C_{23}H_{28}N_2O_6$	Isomajdine	0.9346	alkaloid
15.92	413.38	412.49	$C_{23}H_{28}N_2O_5$	Gardneramine	7.0093	alkaloid
16.50	722.68	721.93	$C_{39}H_{63}NO_{11}$	Solanidine base + *O*-Hex-Hex	4.6729	alkaloid
18.39	576.57	575.31	$C_{32}H_{41}N_5O_5$	Ergocryptine	1.8692	alkaloid

19.34	121.12	120.10	$C_4H_8O_4$	2,3,4-Trihydroxybutanal	0.9346	carbohydrate
20.45	694.65	692.89	$C_{38}H_{60}O_{11}$	Bayogenin base + *O*-HexA	2.3365	saponin

Table S5 FTIR peak values and functional group in ethanolic extract of Black Plum leaf

Sl. no.	Wave number (cm-1)	Intensity	Bond responsible	Functional groups
1	3298.6	strong, broad	C-H stretching	alkyne
2	2972.7	strong, sharp	N-H stretching	amine salt
3	2926.5	medium	O-H stretching	alcohol
4	2880.2	medium	O-H stretching	carboxylic acid
5	2162.8	weak	C≡C stretching	alkyne
6	1457	medium	C-H bending	alkane
7	1376	medium	O-H bending	phenol
8	1263.2	medium	C-O stretching	aromatic ester
9	1114.7	strong	C-O stretching	aliphatic ether
10	1047.2	strong	CO-O-CO stretching	anhydride
11	879.4	strong	C=H bending	1,3-disubstituted
12	577.6	strong, broad	C-Cl stretching	halo compound

Table S6 FTIR peak values and functional group in hexane extract of Black Plum leaf

Sl. no.	Wave number (cm-1)	Intensity	Bond responsible	Functional groups
1	2957.3	strong, sharp	N-H stretching	amine salt
2	2922.6	strong, sharp	O-H stretching	alcohol
3	2858	strong, sharp	C-H stretching	alkane
4	2283.3	weak	O=C=O stretching	carbon dioxaide
5	1602	strong, broad	C-C stretching	conjugated alkene
6	1457	medium, sharp	C-H bending	alkane
7	1378	medium, sharp	O-H bending	phenol
8	1064.5	medium	C-O stretching	primary alcohol
9	972.9	medium	C=C bending	alkene
10	867	weak	C=H bending	1, 3-disubstituted
11	824.4	weak	C=C bending	alkene
12	584.3	strong, broad	C-Cl stretching	halo compound

Figure S1 Plant growth indicators measurement A. SPAD value (chlorophyll content) and B. photosynthesis rate

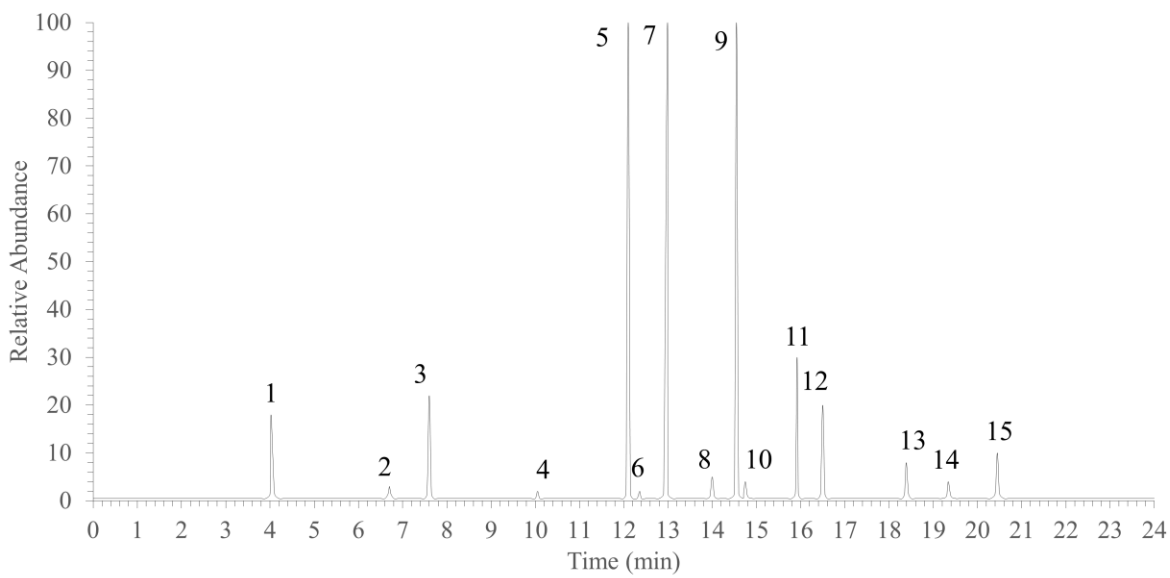
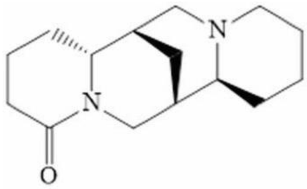
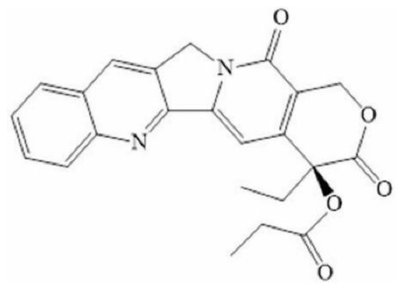
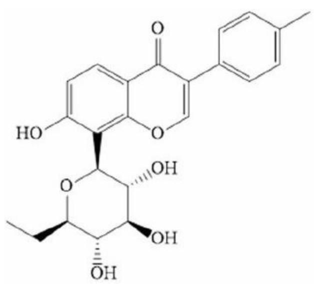


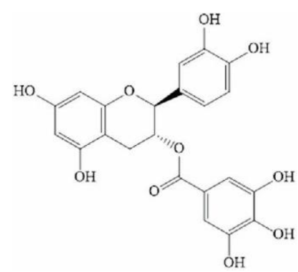
Figure S2 LC–MS chromatogram for the hexane extract of Black Plum leaf





Lupanine **Acetylated camptothecin**





Daidzein-8-*C*-glucoside **Catechin gallate**

Figure S3 Four biopesticide compounds identified by LC-MS from hexane extract of Black plum leaves

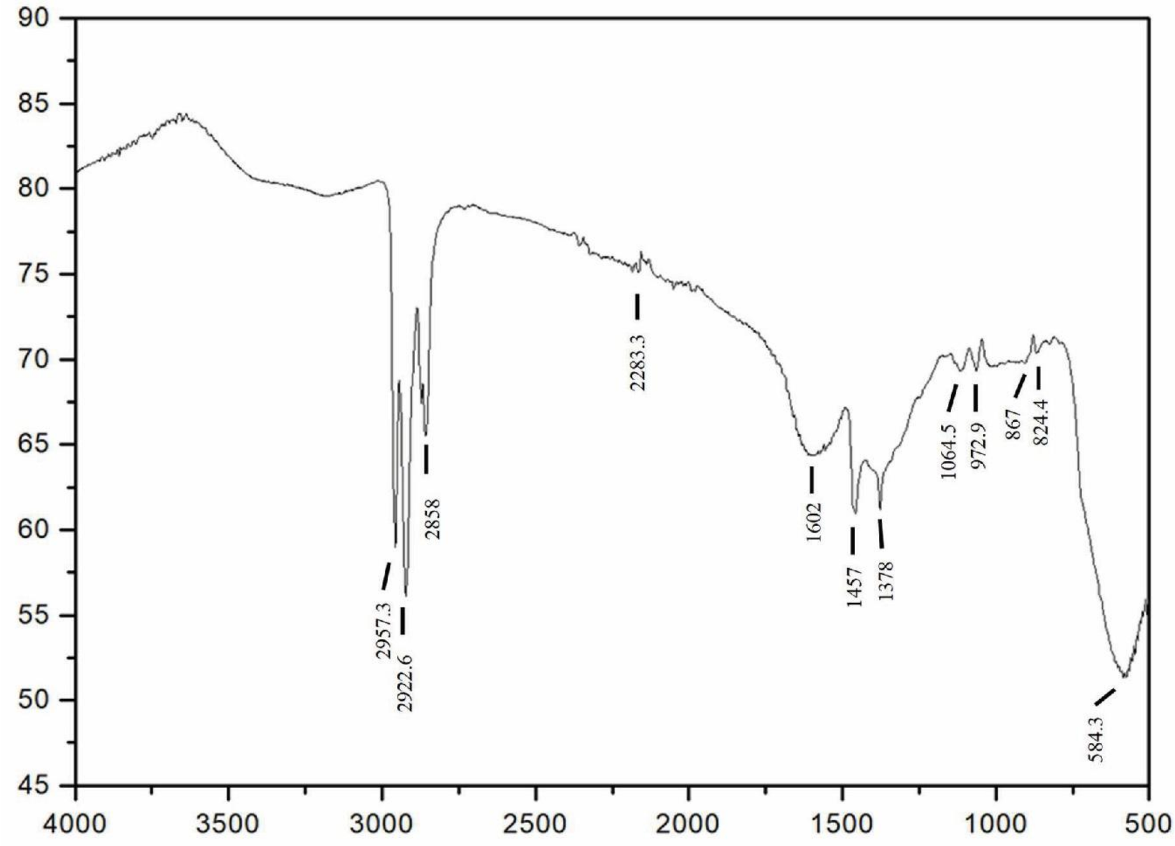


Figure S4 Fourier transform infrared spectrum of the black plum leaf hexane extract