



Identification and Control Model for Pest Organisms in Cabbage Plants

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Received: October 29, 2023

Revised: December 11, 2023

Accepted: December 25, 2023

Published: December 31, 2023

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DOI: [10.29303/jppipa.v9iSpecialIssue.7047](https://doi.org/10.29303/jppipa.v9iSpecialIssue.7047)

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Abstract: The aim of the research is to identify pests according to family and find out the types and concentrations of vegetable pesticides that are effective for controlling pests on cabbage plants using vegetable pesticides, using net sweeping and using light on cabbage plants. This research activity included 2 stages. The first stage is to identify pests on cabbage plants using the insect net method and light lamps, taking insects directly every 7 days of observation. The number of pest insects obtained is recorded and identified based on their family. The second stage is the application of vegetable pesticides and the frequency of spraying on the intensity of pest and disease attacks on cabbage plants. This research is a factorial experimental study using a randomized block design (RAK) method consisting of 2 factors with the first factor being the type of vegetable pesticide consisting of 4 levels, namely (F0) control, (F1) neem, (F2) Annona and (F3) Widuri. The second factor is that the spraying frequency varies, consisting of 3 levels, namely (K1) 3x spraying (K2), 5x spraying and (K3) 7x spraying. Parameters observed included plant height, number of leaves, plant fresh weight, percentage of pest attack intensity and incidence of cabbage disease. Data were analyzed using the Duncan Multiple Range Test (DMRT) 5%. The research results show that the insect pests identified are the families Pyralidae, Plutellidae, Acrididae, Noctuidae and Aphididae. The results of testing a pesticide that is effective in controlling insects is neem pesticide with a spraying time of 7 times which can reduce the intensity of pest and disease attacks on cabbage plants.

Keywords: Cabbage Plants; Diseases; Inventory of Pests; Vegetable Pesticides

Introduction

Cabbage (*Brassica oleracea* L.) is a type of vegetable commodity that has high economic value and is a rich source of vitamins (A, B and C), minerals, carbohydrates, protein and fat which are very useful for health. Every 100 g of boiled or steamed cabbage provides 35 calories, 2.3 g protein, 0.4 g fat, 7.2 g carbohydrates and 3.3 g fiber (Patty, 2018). Cabbage is one type of vegetable that is a mainstay commodity in the North Central Timor Regency (TTU) area. Cultivating this commodity is not free from attacks by pests and diseases, so farmers often experience crop failure (Lanas et al., 2023). In general, farmers in dry land

areas in North Central Timor Regency usually control pests on cabbage plants according to their abilities, either traditionally or using chemical pesticides without paying attention to the dosage, so sometimes the dosage is excessive. This causes resistance in pests, causing pest explosions. Chemical pesticides disrupt or damage the environment, accumulating residues on plants, soil and water, which can harm farmers and consumers. For this reason, plants are needed around farmers' yards or land which can be used as biopesticides.

Organic pesticides are medicinal ingredients to control pests on plants which are made from natural ingredients. Because they are made from ingredients found in nature, this type of pesticide is more

How to Cite:

Nik, N., Rusae, A., Tasekab, O., Bano, F., Pais, Y.O., & Tanii, Y. (2023). Identification and Control Model for Pest Organisms in Cabbage Plants. *Jurnal Penelitian Pendidikan IPA*, 9(SpecialIssue), 1112-1120. <https://doi.org/10.29303/jppipa.v9iSpecialIssue.7047>

environmentally friendly and safer for human health. Organic pesticides have several advantages, including being friendlier to nature, safe for consumption and high economic value. Apart from that, farmers can make organic pesticides themselves. Apart from organic control, it is very important to inventory pests on cabbage plants.

Pest identification in general is making certainty about a pest based on visible symptoms, including other factors related to the attack process (Damiri, 2019). Identification provides information about the types of plant pests and is very important in supporting cultivation implementation, especially determining appropriate control techniques. The district (TTU) is an area with potential for cultivating cabbage, however there is no current information about pests in this area. Knowledge about the presence of pests is very important to determine the pest distribution map as well as to determine further management steps so that it is hoped that it can increase cabbage production.

Method

This research was carried out in Lapeom Village, West Insana District, TTU Regency in July – November 2023. This activity included two testing stages, namely:

Testing for identification of insect pests on cabbage plants. Cabbage plants that have grown are placed in experimental plots, insect pests are taken using insect nets (net sweeping) and installation of light (light traps). The insect net method is carried out by using an insect net during the day and collecting it by swinging the net around the cabbage planting plot. The lamp light method is done by hanging the lamp on a wooden pole, underneath which a container containing detergent is placed. The trapped pest insects are put into an insect bottle containing ethanol, then the number is recorded and then identified according to their family in the laboratory. Observations were made every 7 days.

Testing of Plant Extract Types and Concentrations Against Pest Attacks on Cabbage Plants Tools and materials used in the research include: handsprayer, bucket, water hose, water motor, stirrer, loupe, microscope, stationery and books, gauze, raffia, tractor, crowbar, hoe, machete, meter roll, net insects, lights, PGPR, 70% alcohol and ethanol. Meanwhile, the materials used include vegetable pesticides, manure, liquid fertilizer, cabbage seeds.

The research design used was a factorial Randomized Group Design (RGD) consisting of 2 factors. Factor 1: Type of vegetable pesticide extract consisting of: Control (F0), Neem (F1), Annona leaves (F2) and Widuri leaves (F3). The second factor is the spraying frequency which consists of 3 levels, namely (K1) 3x spraying (K2), 5x spraying, (K3) 7x spraying. Implementation of activities includes:

Land Preparation. Before planting, weeds were first cleaned, then plowed and continued with making beds

measuring 1.5 m x 2 m for 48 experimental units. In the experimental plots, 2 kg of cow manure was given by immersion.

Seed Preparation, Seeding and Planting. Cabbage seeds were obtained from agricultural stores which were free from pests and diseases. Seed sowing is done by soaking the seeds in PGPR solution 50 g/10 liters of water for 30 minutes in a nursery plot measuring 1 x 2 m and first immersing 2 kg of cow manure in the plot then sprinkling cabbage seeds on the surface of the plot and doing this every day. spraying and 2 week old plants were transferred to experimental plots. Planting is carried out in the afternoon with a spacing of 30 cm x 30 cm.

Preparation of Plant Extracts. Neem leaves, annona leaves, thistle leaves, each weighing 3 kg fresh, are dried in the sun until dry, then pounded using a mortar and then sifted to get powder.

Preparation for Application of Vegetable Pesticides. Weigh 50 grams of powder from each plant, then mix it with 1 liter of water, then put it in a handsprayer and spray it on cabbage plants that are attacked by pests. Observation parameters include: Plant height was measured using a 100 cm scale ruler from the underground surface to the top surface of the plant and was carried out at the age of 14 DAT, 28 DAP and 42 DAP. The number of leaves was carried out by counting the number of leaves formed and observations were made at the age of 14 DAP, 28 DAP and 42 DAP. Plant Fresh Weight is carried out by weighing all parts of the plant and expressed in grams (g) of the plant. The fresh weight of the plant is weighed at harvest. Economic Fresh Weight is obtained from parts of plants that are considered economical or parts of plants that can be consumed by separating the parts of the roots and leaves that are not of economic value after which they are weighed and expressed in grams (g). Intensity of pest attacks using the Formula 1.

$$I = \frac{n \times}{N} \times 100\% \quad (1)$$

Information :

I = Attack intensity

n = Number of plants affected

N = Total number of plants observed (Syahrawati & Busniah, 2009) .

Disease incidence is the percentage of plants attacked by pathogens. Cooke (2006) calculates the percentage of disease incidence using the following Formula 1:

$$I = \frac{n}{N} \times 100\% \quad (2)$$

I = Disease Occurrence

n = Number of plants affected

N = Number of all plants observed at each time treatment.

Data analysis used analysis of variance (ANOVA), then tested further using the Dance Multiple Range Test (DMRT) 5%.

Result and Discussion

There was an interaction between neem botanical pesticide treatment and a spraying frequency of 7 times at 28 DAP observations with a height of 16.77 cm. It is assumed that the greater the spraying frequency, the plants will not be disturbed by pest organisms so their growth and development will proceed well, while the first and last observations There was no interaction, but neem vegetable pesticides could increase height increase by 11.29 cm and 22.40 cm respectively.

Number of Leaves

There was an interaction between the neem botanical pesticide treatment and a spraying frequency

of 7 times at 28 DAP observations with a number of leaves of 11.22 pieces, the first observation at 14 DAP also occurred, namely having a greater number of leaves, namely 7.78 pieces. Meanwhile, in the last observation there was no interaction, but the neem vegetable pesticide had a number of leaves of 16.04, while a spraying frequency of 7 times had a number of leaves of 16.33. This is thought to be due to the presence of biotic and abiotic factors in the plant. Availability of nutrients and balanced water increases plant growth and yields. Plant growth is influenced by the water content in the crop/leaves of cabbage plants. According to Kapoor et al. (2020), states that with increasing metabolic productivity, plants will need more nutrients and increase water absorption, this is related to the needs of plants during the growth and development of plants. The application of botanical pesticides controls pest and disease attacks so that plant damage is also low, resulting in increased crop/leaf formation on cabbage plants.

Table 1. Plant Height

Observation time (DAP)	Vegetable Pesticides	Spraying Frequency			Average
		3 times	5 times	7 times	
14	Control	9.67	10.89	12.67	11.08a
	Neem	10.22	11.11	12.55	11.29a
	Annona	10.11	10.77	10.89	10.59a
	Thistle	11.11	10.67	12.00	11.26a
	Average	10.28b	10.86ab	12.03a	(-)
28	Control	12.67b	13.00ab	15.77ab	13.81
	Neem	12.55b	13.67ab	16.77a	14.33
	Annona	11.89b	12.44b	13.67ab	12.67
	Thistle	12.11b	13.33ab	14.11ab	13.18
	Average	12.31	13.11	15.08	(+)
42	Control	19.11	19.78	23.33	20.74a
	Neem	20.33	22.11	24.77	22.40a
	Annona	19.00	21.22	21.22	20.48a
	Thistle	20.55	20.89	22.89	21.44a
	Average	19.75b	21.00ab	23.05a	(-)

Note: Numbers in rows and columns followed by the same letter indicate no difference at the 5% significant level (α) according to DMRT; (-): There is no interaction between factors. (+): there is an interaction between factors.

Plant Fresh Weight

There was an interaction between the neem botanical pesticide treatment with a spraying frequency of 7 times which had a fresh plant weight of 1,217.88 g. Environmentally friendly plant pesticides have an impact on improving the physics, biology and chemistry of the soil so that it becomes a good growing medium for plant growth and yields. The application of botanical pesticides reduces the activity of pests and pathogens so that they do not interfere with plant growth. Botanical pesticides can kill or disrupt pest and disease attacks through a unique way of working, namely through a combination of various methods or singly. The way

botanical pesticides work is very specific, namely; damage the development of eggs, larvae and pupae; inhibits skin turnover; disrupt insect communication; causes insects to refuse to eat; inhibits insect reproduction; repels insects; inhibits the development of disease pathogens, this is in accordance with the statement Zahro et al. (2016) which states that the feeding activity of *P. xylostella* larvae is reduced due to disruption of the body's nervous system and metabolic system due to the presence of secondary metabolite compounds in lemongrass extract in the feed. This shows that neem extract can quickly control pests and diseases.

Table 2. Number of leaves

Observation time (DAP)	Vegetable Pesticides	Spraying Frequency			Average
		3 times	5 times	7 times	
14	Control	4.78b	5.11b	7.22a	5.89
	Neem	4.89b	5.67ab	7.78ab	5.93
	Annona	5.00b	5.00b	6.11ab	5.37
	Thistle	5.00b	5.00b	5.87ab	5.29
	Average	4.92	5.20	6.75	(+)
28	Control	7.99ab	8.66ab	10.45ab	9.03
	Neem	8.11ab	9.77ab	11.22a	9.70
	Annona	9.44ab	8.44ab	8.77ab	8.88
	Thistle	7.55b	7.89b	10.44ab	8.63
	Average	8.27	8.69	10.22	(+)
42	Control	13.77	14.66	17.00	15.14a
	Neem	14.11	16.33	17.67	16.04a
	Annona	13.89	15.22	13.77	14.29a
	Thistle	14.22	14.55	16.87	15.21a
	Average	14.00b	15.19ab	16.33a	(-)

Note: Numbers in rows and columns followed by the same letter indicate no difference at the real level (α) of 5% according to DMRT; (-): There is no interaction between factors. (+): there is an interaction between factors.

Table 3. Fresh Weight of Plants

Vegetable Pesticides	Spraying Frequency			Average
	3 times	5 times	7 times	
Control	831.62b	795.47b	1188.33a	938.47
Neem	828.33b	769.92b	1217.88a	938.71
Annona	927.52ab	933.55ab	794.94b	885.34
Thistle	1106.40ab	1034.00ab	985.74ab	1042.05
Average	923.47	883.24	1046.72	(+)

Note: Numbers in rows and columns followed by the same letter indicate no difference at the 5% significant level (α) according to DMRT; (+): there is an interaction between factors.

Economic Fresh Weight

The results of the anova test showed that there was an interaction between the neem vegetable pesticide treatment with a spraying frequency of 7 times which had an economic fresh weight of 865.81 g which was not significantly different from the other treatments. Neem botanical pesticide treatment and the right frequency provide protection to plants, the pesticide is able to suppress pest and disease activity so that plants are not disturbed by pest attacks, plants grow optimally according to their growth age. The nature of botanical pesticides is that they are toxins that are not liked by pests and diseases. This is supported by (Hasyim et al., 2014; Musyadah et al., 2015) stated that the toxicity of babadotan leaf extract, neem is undesirable to fall armyworms and *Plutella xylostella* caterpillars, thereby suppressing their growth and development, preventing eating, inhibiting communication, and disrupting metamorphosis. This will have a positive effect on the economic fresh weight of cabbage plants.

Table 4. Economic Fresh Weight.

Vegetable Pesticides	Spraying Frequency			Average
	3 times	3 times	3 times	
Control	530.00c	521.52c	809.69ab	620.40
Neem	517.12c	487.48c	865.81a	623.47
Annona	564.20bc	529.18c	522.66c	538.68
Thistle	686.08abc	633.41abc	658.39abc	659.29
Average	574.35	542.90	714.14	(+)

Plutella xylostella Pest Attack Intensity (%)

The intensity of the pest attack by P. The intensity of these pest attacks will decrease because spraying is carried out several times. The more frequency of spraying, it is thought that the level of pest attacks on plants will decrease, thereby suppressing the development of pests. The results of research by Nik (2023), showed that At nonse (*Annona muricata L.*) powder extract suppressed the growth and development of *Sitophilus sp* imago in storage, causing the pest's body condition to become weaker and resulting in a decrease in appetite so that the pest would experience death quickly. Neem botanical pesticide has a working mechanism as an antifeedant, growth inhibitor, and reproductive disturbance in *D. Melanogaster* (Bezzar-Bendjazia et al., 2016) and *S. frugiperda* (Duarte et al., 2020).

Note: Numbers in rows and columns followed by the same letter indicate no difference at the 5% real level (α) according to DMRT; (+): there is an interaction between factors.

Table 5. Intensity of pest attacks (%)

Observation time (DAP)	Vegetable Pesticides	Spraying Frequency			Average
		3 times	3 times	3 times	
14	Control	21.03c	22.44d	21.78cd	21.75
	Neem	12.22b	11.14ab	10.37a	12.22
	Annona	13.62b	13.44b	12.67b	13.24
	Thistle	11.74ab	13.78c	12.03b	12.52
	Average	14.65	16.55	15.49	(+)
28	Control	20.22d	21.03d	22.55e	21.27
	Neem	11.81ab	10.62ab	10.55a	10.99
	Annona	12.22ab	11.62ab	12.15ab	12.00
	Thistle	11.62ab	11.85ab	12.62c	12.03
	Average	13.97	13.78	14.47	(+)
42	Control	18.51c	19.62d	22.44e	20.19
	Neem	10.81ab	11.22ab	10.44a	10.82
	Annona	13.51b	12.22b	10.74ab	12.16
	Thistle	12.62b	10.74ab	12.92b	12.09
	Average	13.86	13.45	14.33	(+)

Note: Numbers in rows and columns followed by the same letter indicate no difference at the 5% real level (α) according to DMRT; (+): there is an interaction between factors.

Disease Incidence (%)

There was an interaction between neem botanical pesticide treatment and a spraying frequency of 7 times which was able to reduce the incidence of disease by 14.81%. Based on the results of observations on the research land, there were no diseases caused by pathogens, only diseases caused by abiotic factors. At the age of 42 DAP, the leaves of the plant become purplish, starting from the first leaf and progressing to the next leaf. This symptom is thought to be a lack of the element phosphorus (P). Widarti et al. (2015) stated that the symptoms of a lack of P levels in plants are greatly reduced root growth, old leaves turning purplish and yellowing prematurely and stunted plants. The element phosphorus (P) as an organic material has a very important role in soil fertility, photosynthesis processes and chemical physiology of plants. Phosphorus is also needed in cell division, tissue development and plant growth points. Neem pesticide treatment is effective in

controlling diseases in cabbage plants. The various types of pesticides given greatly influence the level of disease incidence, so they also influence the size of the intensity of the damage they cause. Environmentally friendly plant pesticides have an impact on improving the physics, biology and chemistry of the soil so that it becomes a good growing medium for plant growth and yields. When controlling/without spraying, the intensity of disease attacks is quite high, causing the quality and quality of cabbage plants to decrease, causing a high level of yield loss. Oka (2015) stated that aphids are pests that can reduce the quality and quantity of plant production and are an important vector that can transmit viral diseases to plants. Aphids usually attack under the surface of the leaves or between the leaves, sucking fluids. young leaves, petioles and stems, which causes plant growth to be disrupted and crop production decreases.



(a)



(b)

Figure 1. Cabbage Plants: (a) Diseases caused by temperature; and (b) Diseases caused by nutrients P

Table 6. Disease Incidence

Observation time (DAP)	Vegetable Pesticides	Spraying Frequency			Average
		3 times	3 times	3 times	
42	Control	44.44a	29.62abc	18.51bc	30.86
	Neem	44.44a	22.22abc	14.81c	27.16
	Annona	40.74ab	22.22abc	18.51bc	27.16
	Thistle	25.92abc	40.74ab	29.62abc	32.09
	Average	38.89	28.70	20.36	(+)

Note: Numbers in rows and columns followed by the same letter indicate no difference at the 5% significant level (α) according to DMRT; (+): there is an interaction between factors.

Installation of lights (light trap insects)

In the light trap insect treatment, *Agrotis ipsilon* imago, *Spodoptera litura* imago, *Crociodolomia binotalis* imago and *Plutella xylostela* imago were found, as well as *Locusta migratoria*, L. lay eggs on cabbage plants, and then they will hatch into larvae that will damage the surface of the leaves of cabbage plants. This is supported by Ashik-E-Rabbani et al. (2022) who stated that insects that are attracted to the light will approach and fall into the trap. Apart from that, insects at night are attracted to light because insects have sensors that are sensitive to high light intensity. Insect vision is influenced by the intensity of light around them, thus affecting the presence of insects around them (Kurniawati et al., 2021; Sihombing et al., 2013).

Observation results show that insect pests attack at the age of 30 DAP to 65 DAP. The number of pests collected using the light trap insect method (804). The intensity of light will affect the presence of night insects trapped in the insect light trap. The light emitted has an intensity that attracts insect pests, thereby influencing the behavior of insects to lay their eggs. This is supported by (Al Mamun et al., 2023; Al Mamun et al., 2023) who said that the light attracts insects.

Use of Net Sweeping

The number of pest insects caught in the net sweeping was (1,518). The types of insects netted were the Pyralidae family, Lepidoptera order, which is thought to be the species *Crociodolomia binotalis*, the Plutellidae family, Lepidoptera order, which is thought to be the species *Plutella xylostella*, and the Aphididae family, Homoptera order, which is thought to be the species *Aphis* sp. Of the two treatments used in this activity, the pests collected in the net sweeping treatment had a higher population than the light trap insect method. It is suspected that this netting was carried out in the morning until the evening, which indicates that insect mobilization is more active in moving to find its host. achieve survival. The use of nets that can be used repeatedly during the cabbage planting period will reduce pest populations and can minimize ongoing costs (Nurfajriani et al., 2022).

The results of the inventory and identification of net sweeping and light trap insect treatments found several (5 families) in cabbage plantings including:

Family Pyralidae (Lepidoptera)

The early instar larvae are greenish yellow in color and live in clusters under the leaves, while the final instars are found in the crop which is damaged to the point of having holes. Symptoms of this damage are thought to be *Crociodolomia binotalis* caterpillars. In young plants the larvae are found in clusters, if the plant gets bigger and has formed a crown it will damage the crown with symptoms of irregular holes in the crown. *Crociodolomia binotalis* attacks plants at the age of 46 DAP, attacks cabbage leaves by eating the leaves, the leaves have holes. Hi, this is in accordance with what Narayanasamy (2001) found for crop caterpillars. *C. binotalis* can attack cabbage leaves. Early instar larvae eat leaves and leave the epidermis layer which then develops holes after the epidermis layer dries.

Family Acrididae

This pest attacks cabbage plants at the age of 35 DAP. Symptoms of pest attacks can be seen from bite marks starting at the edges of the leaves until they have holes or tears and severe attacks only leave the bones of the leaves. Adenka et al. (2021); Hanifah & Kusumah (2020) stated the symptoms of an attack seen in leaves that are hollow and shriveled. In more severe damage, the leaves will turn yellow, leaving only the bones of the leaves due to pest bite marks and the leaves will wilt.

Family Plutellidae

Observations in cabbage plantations showed that the larvae were green and their heads were black and their hairs grew straight. The surface tissue of the leaves is eaten, causing transparent spots to form white spots. The 3-5th instar larvae eat the leaves until they have holes. These characteristics show that the destructive caterpillar is *Plutella xylostela*. *P. xylostela* larvae usually eat young plants to plants that have already formed crops and attack the leaves. Sembel (2014) stated that newly hatched larvae are greenish in color and have black heads. These *P. Xylostela* larvae actively eat leaves until they have holes. *P. xylostela* is a polyphagous pest insect, namely an insect that attacks plant species from the same family, both cultivated plants and wild plants (weeds) (Hendriwal & Khaidir, 2012).

The Plutellidae family in the Net sweeping treatment has a higher population than in the Light trap

insect treatment. The mature larvae (4-5th instar) will damage the crop parts of the plants and leave dirt

residue on the crop. Fifi (2022) stated that the cabbage crop appeared to have holes.

Table 7. Population of Pest Insects When Using Net Sweeping and Installing Lights

Treatment	P1	P2	P3	P4	P5	P6	Total
Day after plant	30	37	44	51	58	65	
Net Sweeping	76	153	249	266	358	416	1.518
Family							
Pyralidae	20	42	89	96	155	199	
Plutelidae	8	30	65	68	79	83	
Acrididae	7	8	8	12	34	15	
Noctuidae	21	38	32	25	30	29	
Aphididae	20	35	55	65	60	90	
Ligth traps	58	103	144	161	230	299	995
Family							
Pyralidae	14	23	55	66	73	112	
Plutelidae	16	12	25	54	65	75	
Acrididae	9	4	0	3	11	0	
Noctuidae	4	39	19	5	52	68	
Aphididae	15	25	45	33	29	44	



Figure 2. *Plutella xylostella* larvae

The attack is most damaging when the plant is still young or at the budding stage. Moths do not cause direct damage to the buds, but damage the wrapping leaves, although they do not directly affect crop yields, they can reduce the value of the crop (Manikome, 2021).

Family Noctuidae

Armyworm (*Spodoptera litura* F.) from the Noctuidae family is an important pest on crops, cabbage and mustard greens. This pest has polyphagous properties so it can eat various types of plants for its survival. The small larvae damage the leaves by biting the leaves until they use up the green leaf substance (transparent) and only the leaf bones remain. Later instar larvae damage the leaf veins and sometimes attack the crop. Usually the larvae are on the lower surface of the leaves, and attack simultaneously in groups. Armyworms (*Spodoptera litura*) belong to the order Lepidoptera. This pest is polyphagous, so it is quite difficult to control. Symptoms of *S. litura* attack start from the larval stage, where the young larvae damage the leaves and leave traces of the remains of the upper

(transparent) epidermis and leaf veins. Later instar larvae damage the leaf veins, and cause the plant to lose leaves. Symptoms of attacks that are fast and difficult to control cause the need for intensive control (Marwoto, 2008).

Aphididae family

Aphids *Brevicoryne brassicae* (cabbage aphids) are the main species that attack cabbage, aphids attack cabbage plants at 15 DAP sucking leaves, and young stems, the next symptoms are that the affected leaves become wrinkled, yellowish, twisted, plant growth is stunted, wilts and then dies. Sista (2016) stated that aphid attacks cause young shoots and leaves to curl, the leaves curl silver and even turn brown and then die. These pests are leaf-sucking pests and are difficult to control because they are polyphagous (Meilin, 2014).

Conclusion

The disease that attacks cabbage plants is a disease caused by abiotic factors, namely a lack of the nutrient P and very high temperatures causing wilting, yellowing and drying of the leaves. The pests identified are the families Pyralidae, Plutelidae, Acrididae, Noctuidae and Aphididae. The type of pesticide that is effective in controlling pests and diseases is Neem vegetable pesticide with a spraying frequency of 7 times and the Net Sweeping method can capture large pest populations.

Acknowledgments

Thank you to the LPPM University of Timor for funding and supporting the implementation of this research so that it can be completed well and smoothly.

Author Contributions

This article was prepared by six authors, namely N.K, A.R, O.T, F.B, Y.O.P, and Y.T. All authors members worked together at every stage of preparing this article.

Funding

This research received funding from LPPM of Timor University.

Conflicts of Interest

The authors declare no conflict of interest.

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