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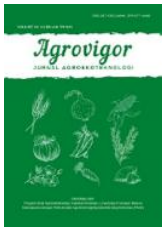

Article

The Effect of Chitosan on Enhancing the Defense Mechanisms of Yard Long Bean Plants Against *Aphis craccivora* Koch.

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ABSTRACT

Yard-long bean production in Indonesia has declined due to environmental factors and pest infestations, particularly by *Aphis craccivora*, a vector of viruses such as *Bean common mosaic virus* (BCMV). Generally, *A. craccivora* has been controlled using insecticides; however, their use poses limitations including toxicity, the development of resistance, environmental concerns, and disruption of pest–predator dynamics due to excessive application. As a promising alternative, chitosan has demonstrated potential in inhibiting aphid feeding, reducing reproduction rates, delaying disease incubation periods, and lowering BCMV titres. This study assessed the effects of both pure and commercial chitosan on *A. craccivora* in yard-long beans. The evaluation focused on antixenosis, antibiosis, and insecticidal properties. The results showed that chitosan significantly reduced aphid colonisation and feeding preference, lowered infestation intensity, and enhanced natural predation. Furthermore, chitosan treatments suppressed aphid reproduction, prolonged the aphid life cycle, and decreased their growth rate. The direct spray method was found to be more effective than the systemic application. Among the treatments, KK 0.9 consistently produced the most favourable outcomes across all parameters, indicating its potential as an effective bio-insecticidal agent for pest and disease management in yard-long bean cultivation.

INTRODUCTION

Yard-long beans (*Vigna sinensis* (L.) Savi *ex* Hassk) are plants belonging to the Fabaceae family that have been utilized and cultivated by the Indonesians for harvesting their young pods (Wahyuni et al., 2021). Yard-long beans have quite complex nutritional content, including iron, protein, fat, phosphorus, carbohydrates, calcium, and crude fiber (Kaswinarni et al., 2014; Khatun et al., 2022), with vitamin A, vitamin B-6, vitamin C, and other micronutrients (USDA, 2019). National yard-long bean production has experienced a significant decline over the years. In 2021, yard-long bean production reached 383,685 tons, but in 2022, it continued to decline to 360,871 tons, and by 2023, production had further decreased to 309,422 tons (BPS, 2024).

The factors contributing to the decline in yard-long bean production are complex, including environmental factors and pest or pathogen attacks. Pests commonly found in yard-long bean plantations include *Paraeuscosmetus pallicomis*, *Aphis craccivora*, *Oxya* sp., and *Spodoptera* spp., while diseases that commonly infect include *Mungbean Yellow Mosaic Virus* (MYMV) and *Bean Common Mosaic Virus* (BCMV) (Murwarni et al., 2022). MYMV has been reported in North Sumatra (Musi Rawas), with a field incidence of 30%–50% showing yellowing symptoms (Sutrawati & Aulia, 2024). Damayanti et al. (2009) reported an outbreak of the disease in several areas in West Java (Bogor, Bekasi, Indramayu, and Cirebon), characterized by yellow mosaic symptoms associated with the BCMV virus strain *Black-eye Cowpea* (BCMV-BIC). The severity of the disease in the field can reach up to 100%, with a decrease in yield ranging from 27.1% to 85.2%. Control is challenging due to the nature of seed and vector transmission.

One of the virus vectors in yard-long bean plants is *A. craccivora* Koch (Hemiptera: Aphididae). Transmission of the virus through *A. craccivora* is non-persistent, meaning that the virus survives in the stylet for only a short time (Megasari et al., 2014). *Aphis craccivora* is brown to blackish in color and lives in colonies on shoots, young leaves, flowers, old leaves, and pods. The feeding activity of aphids in high populations causes leaf malformation, reduced production, stunted growth, distorted plant shape, and inhibition of plant development (Irsan et al., 2023).

Aphid control has been implemented through various methods, including sanitation, the use of resistant varieties, enhancing host plant resistance, and the application of pesticides. Pesticides are a widely used control method due to their ability to cause rapid mortality. The use of pesticides has side effects, including toxicity to mammals, non-target organisms, and environmental pollution. Additionally, the cost of using pesticides is relatively high. One of the impacts of excessive fertilizer and pesticide use is an imbalance in pest and predator populations, leading to an overpopulation of pests (John & Babu, 2021). Another impact is the emergence of more resistant pests, resulting from the adaptability of pests and the selection of pesticide-resistant genes (Meray et al., 2024).

The introduction of new food crop varieties in the mid-20th century led to a significant increase in the production of cereals such as rice and wheat, a phenomenon known as the Green Revolution. These new varieties require excessive agricultural inputs, including large amounts of chemical fertilizers and pesticides (Britannica, 2024). Excessive use of inputs raises concerns about environmental sustainability. Continuous use of resistant varieties in monoculture planting can lead to changes in biological features, resulting in the development of new insect biotypes that can overcome plant resistance (Cheng et al., 2013). According to Khanal et al. (2023), many new aphid biotypes have been identified based on their biological features, which allow them to overcome the resistance of previously resistant host plant species or varieties. These biological features indicate changes in feeding preferences or behavior to obtain nutrients. In addition to breaking the host plant's resistance, these changes can also expand the host range.

Currently, physiologically active compounds are needed as alternatives in pest control. These compounds must be highly selective to avoid negative impacts on non-target organisms and the environment. Physiologically active compounds are expected to enhance plant resistance by increasing the activity of resistance-inducing genes. Chitosan is considered a promising control alternative due to its broad spectrum, non-toxicity to non-target organisms, including mammals, and its biodegradability (Abourehab et al., 2022; Kholiq & Kusuma, 2024). Chitosan acts as a protein inducer, enhancing plant resistance to pathogens. It also serves as a growth trigger, boosting plant immunity and defense against pathogens (Maluin

& Hussein, 2020). Chitosan plays a role in inhibiting the development of microorganisms and exhibits antifungal properties (Suryadi et al., 2020). It also inhibits aphid feeding and demonstrates insecticidal activity, as well as antixenosis and antibiosis effects, although these effects are not permanent (Adiwena et al., 2021; Megasari et al., 2014). Chitosan is known to cause mortality in *A. nerii* on oleander plants and *Spodoptera littoralis* on cotton plants in Egypt through its insecticidal activity (Badawy & El-Aswad, 2012). Research by Megasari et al. (2019) demonstrated that chitosan inhibits *A. craccivora* feeding and can suppress disease incidence, extend the incubation period, reduce disease severity, and lower BCMV titer values in yard-long bean plants. This study aims to evaluate the effects of antixenosis, antibiosis, and insecticidal activity of chitosan on *A. craccivora*.

MATERIALS AND METHODS

The research was conducted at the IPB Cikabayan Dramaga Experimental Garden Greenhouse and the Plant Virology Laboratory, Department of Plant Protection, IPB University. Aphids are reared on taro leaves (*Colocasia esculenta* (L.) Schott), with the leaf stalk tips wrapped in wet cotton. Apterous imagos are placed in a petri dish containing taro leaves and maintained until they give birth to nymphs. The long beans used are of the Parade variety. The seeds are planted in 30×35 cm polybags filled with a mixture of sterile soil and manure in a 2:1 ratio.

Pure chitosan (Biobasic), derived from crab shells ($C_6H_{11}NO_4$) with a purity of 90%, was used in this study. For comparison, commercial chitosan (Soft Guard Chitosan Oligo Saccharin) with a chitosan content of 2% was also tested. The concentrations of pure chitosan used were 0.1%, 0.3%, 0.5%, 0.7%, 0.9%, and 1.1% (KM 0.1-1.1), while the commercial chitosan was used at a concentration of 0.9% (KK 0.9). Pure chitosan was diluted with 1.5% acetic acid and sterile distilled water, while commercial chitosan was diluted with only sterile distilled water. Control plants were sprayed with 1.5% acetic acid.

Testing the Antixenosis Effect of Chitosan

Plants aged 2 Weeks After Planting (WAP) were treated by spraying a solution of 3 ml of chitosan per plant, with 10 replications for each treatment. Plants treated with chitosan are placed in gauze cages, arranged randomly in a circular pattern. A white cardboard sheet was placed under the plant canopy. Twenty-four hours after treatment, 50 Apterous imagos were introduced to the center of the plant circle. Aphid colonization was observed at 24, 48, and 72 hours after infestation.

Testing the Antibiosis Effect of Chitosan

Plants aged 2 WAP were treated by spraying a solution of 3 ml of chitosan per plant, with 10 replications for each treatment. The plants are covered with plastic to prevent infestations by other insects. Twenty-four hours after treatment, each plant in the treatment group was infested with an apterous imago. The imago is observed until it gives birth to nymphs (first filial). The nymph that was born first, then one was left and the others were discarded. The remaining nymph is kept and observed until it matures into an imago and gives birth to another nymph (second filial). The nymphs that were born first from the second generation, then one was left and the others were thrown away. One of the remaining nymphs is kept and observed until it matures into an imago, gives birth to nymphs (third filial), and eventually dies. Observations were made on the length of nymph development, pre-viviparity period, life cycle, reproductive period, longevity, and the number of nymphs born (Zeng et al., 1994). Multiplication rate (MR) and Intrinsic Growth Rate (rm) are calculated using the formula:

$$MR = \frac{number\ of\ nymphs\ born}{lifespan\ of\ the\ imago} \quad (1)$$

(Kashyap et al. 1988)

$$r_m = \frac{0.738\ (log\ of\ viviparity\ period)}{generation\ time} \quad (2)$$

(Wyatt & White 1977)

Testing the Insecticidal Activity of Chitosan by Direct Spray Method

Plants aged 2 WAP were infested with 50 second instar nymphs. The aphid nymphs were then sprayed directly onto their bodies with either a pure chitosan solution or a commercial chitosan solution, depending on the treatment, at a rate of 3 ml of chitosan per plant, with 10 replications for each treatment. The plants are covered with plastic to prevent infestations by other insects. Observations were made on the number of aphid mortalities at 24, 48, and 72 hours post-treatment.

The Lethal Concentration ($LC_{50, 95}$) was calculated using Probit analysis (Finney, 1971).

Testing the Insecticidal Activity of Chitosan by Systemic Method

Plants aged 2 WAP were treated by spraying a solution of 3 ml of chitosan per plant, with 10 replications for each treatment. The plants are covered with plastic to prevent infestations by other insects. Twenty-four hours after treatment, each plant in the treatment group was infested with 50 second instar nymphs. The plants are covered with plastic to prevent infestations by other insects. Observations were made on the number of aphid mortalities at 24, 48, and 72 hours post-treatment. The Lethal Concentration ($LC_{50, 95}$) was calculated using Probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Antixenosis Effect on Aphids

Chitosan treatment significantly affected the colonization and feeding preferences of aphids. Aphid colonization was notably lower in treated plants compared to the control, with differences observed as early as the first observation. The average number of aphids on treated plants ranged from 1 to 10 per plant, while in the control group, the average number of aphids reached 19. The highest level of colonization inhibition was observed in the KK 0.9 treatment (Figure 1).

Chitosan sprayed on the leaf surface is believed to exert an antixenotic effect on aphids. Antixenosis is caused by morphological or biochemical factors in plants that negatively affect the acceptance of the plant as a host (Roddee et al., 2024). Antixenosis can be evaluated through the reduction in the number of pest colonizations (de Oliveira et al., 2023). Antixenosis is a key component of plant resistance to aphids, as it can prevent or delay aphid colonization and reduce the potential for infestation that may reach economic thresholds. By preventing aphid colonization, it effectively manages the initial aphid population and limits the number of offspring produced. Aphids that are deterred from settling on chitosan-treated yard-long bean plants are forced to continue searching for suitable hosts, which increases the likelihood of them being preyed upon by natural predators before finding a suitable plant.

Antibiosis Effect on Aphids

Chitosan treatment is thought to have an antibiosis effect on the reproduction of aphids, this can be seen based on the number of first nymphs born by aphid imago infested on treatment plants which is significantly lower when compared to the control (Table 1). Antibiosis is a mechanism that causes negative impacts on the life cycle of pests due to biochemical components contained in the host plant.

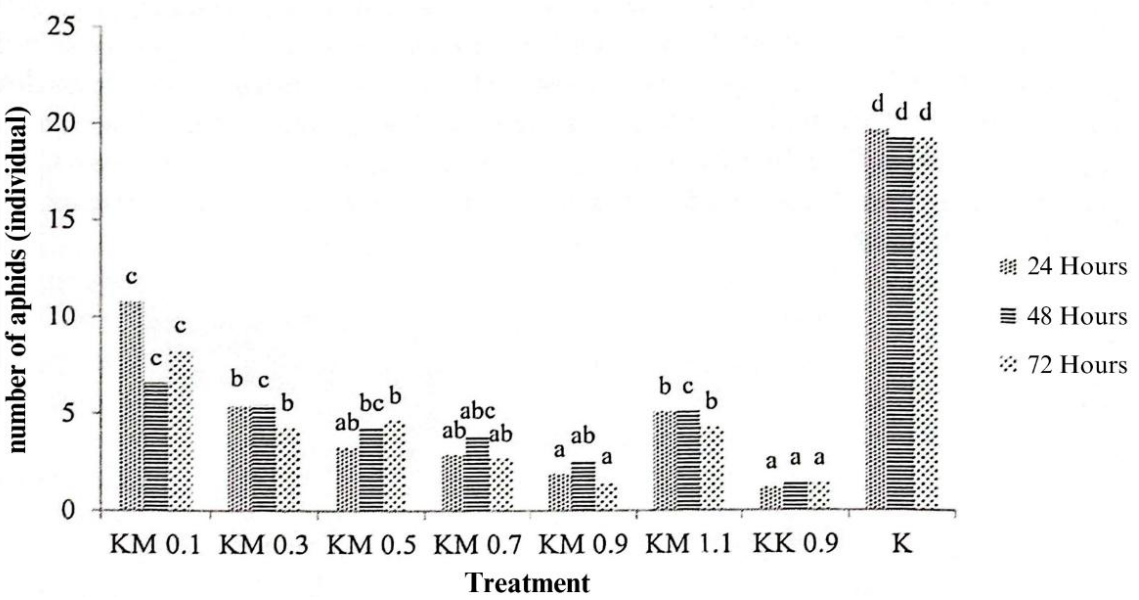

Figure 1. Effect of treatment on aphid colonization.

Table 1. Biology of first generation aphids in chitosan treatment

Treatment	Number of 1st Instar Nymphs	1st instar	2nd instar	3rd instar	4th instar	Pre-viviparity	Life cycle
KM 0.1	7.1 ± 1.3 bc	1.0 ± 0.0 a	1.2 ± 0.4 ab	1.0 ± 0.0 a	1.0 ± 0.0 a	0.3 ± 0.4 a	4.5 ± 0.5 a
KM 0.3	5.7 ± 1.5 ab	1.0 ± 0.0 a	1.6 ±0.6 b	1.5 ± 0.7 bc	1.0 ± 0.0 a	0.4 ± 0.5 a	5.5 ± 0.8 b
KM 0.5	7.0 ± 1.3 bc	1.0 ± 0.0 a	1.0 ± 0.0 a	1.2 ± 0.4 abc	1.1 ± 0.3 a	0.4 ± 0.5 a	4.7 ± 0.6 a
KM 0.7	7.3 ± 1.3 c	1.0 ± 0.0 a	1.1 ± 0.3 a	1.1 ± 0.3 ab	1.2 ± 0.4 a	0.3 ± 0.4 a	4.7 ± 0.4 a
KM 0.9	6.4 ± 2.1 abc	1.0 ± 0.0 a	1.2 ± 0.4 ab	1.6 ± 0.5 c	1.0 ± 0.0 a	0.2 ± 0.4 a	5.0 ± 0.4 ab
KM 1.1	7.4 ± 1.5 c	1.0 ± 0.0 a	1.2 ± 0.4 ab	1.2 ± 0.6 abc	1.0 ± 0.0 a	0.3 ± 0.4 a	4.7 ± 0.8 a
KK 0.9	5.2 ± 1.0 a	1.0 ± 0.0 a	2.6 ± 0.8 c	2.6 ± 0.6 d	1.3 ± 0.3 a	0.3 ± 0.4 a	7.8 ± 1.1 bc
K	8.9 ± 1.7 d	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	0.6 ± 0.5 a	4.6 ± 0.5 a

* Numbers followed by different quality letters in the same column indicate significantly different results (DMRT α=0.05)

Table 2. Biology of second generation aphids in chitosan treatment

Treatment	Number of 1st Instar Nymphs	1st instar	2nd instar	3rd instar	4th instar	Pre-viviparity	Life cycle	Logevity
KM 0.1	6.1 ± 0.5 b	1.0 ± 0.0 b	1.1 ± 0.3 a	1.0 ± 0.0 a	1.5 ± 0.5 ab	0.3 ± 0.4 a	4.9 ± 0.7 a	12.2 ± 0.6 b
KM 0.3	6.4 ± 0.5 b	1.0 ± 0.0 b	1.0 ± 0.0 a	1.0 ± 0.0 a	1.7 ± 0.4 b	0.4 ± 0.5 a	5.1 ± 0.8 a	13.1 ± 1.2 b
KM 0.5	6.3 ± 0.4 b	1.0 ± 0.0 b	1.0 ± 0.0 a	1.0 ± 0.0 a	1.2 ± 0.4 ab	0.3 ± 0.4 a	4.5 ± 0.8 a	11.8 ± 1.0 b
KM 0.7	6.1 ± 0.5 b	1.0 ± 0.0 b	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	0.1 ± 0.3 a	4.1 ± 0.3 a	11.5 ± 0.7 b
KM 0.9	6.0 ± 0.6 b	1.0 ± 0.0 b	1.0 ± 0.0 a	1.4 ± 0.5 b	1.1 ± 0.0 a	0.5 ± 0.5 a	4.9 ± 0.7 a	12.0 ± 1.1 b
KM 1.1	6.1 ± 0.7 b	1.0 ± 0.0 b	1.0 ± 0.0 a	1.0 ± 0.0 a	1.1 ± 0.3 a	0.2 ± 0.4 a	4.3 ± 0.6 a	11.7 ± 1.2 b
KK 0.9	2.9 ± 3.0 a	0.8 ± 0.4 a	1.2 ± 0.9 a	0.9 ± 0.5 a	1.4 ± 1.2 ab	0.2 ± 0.4 a	4.5 ± 2.8 a	8.2 ± 4.5 a
K	7.1 ± 0.5 b	1.0 ± 0.0 b	1.0 ± 0.0 a	1.0 ± 0.0 a	1.1 ± 0.0 a	0.5 ± 0.5 a	4.5 ± 0.5 a	12.9 ± 0.9 b

* Numbers followed by different quality letters in the same column indicate significantly different results (DMRT α=0.05)

Table 3. Total fecundity and daily fecundity of the second generation aphid imago

Treatment	Total Fecundity	Daily Fecundity						
		1	2	3	4	5	6	7
KM 0.1	55.9 ± 8.0 b	12.1 ± 3.4 b	11.5 ± 3.2 c	10.1 ± 2.9 b	11.1 ± 1.6 bc	9.7 ± 3.9 b	1.4 ± 2.9 ab	0.0 ± 0.0 a
KM 0.3	53.9 ± 5.6 b	10.2 ± 2.9 b	11.3 ± 1.7 c	9.5 ± 2.0 b	9.4 ± 3.6 b	9.5 ± 2.1 b	4.0 ± 5.4 b	0.0 ± 0.0 a
KM 0.5	46.9 ± 9.8 b	8.8 ± 1.9 b	8.1 ± 2.7 b	7.8 ± 2.9 b	9.0 ± 2.9 b	9.5 ± 0.7 b	3.8 ± 4.9 ab	0.0 ± 0.0 a
KM 0.7	47.9 ± 5.5 b	10.0 ± 2.4 b	9.3 ± 1.4 bc	9.4 ± 1.9 b	9.1 ± 2.4 b	7.3 ± 3.0 b	2.8 ± 4.5 ab	0.0 ± 0.0 a
KM 0.9	49.9 ± 8.2 b	11.7 ± 3.9 b	11.2 ± 3.4 c	9.4 ± 1.4 b	10.0 ± 1.6 b	7.9 ± 4.3 b	1.9 ± 4.0 ab	0.0 ± 0.0 a
KM 1.1	50.4 ± 12.6 b	11.8 ± 5.4 b	10.0 ± 4.2 bc	9.3 ± 3.1 b	9.1 ± 1.1 b	8.2 ± 3.9 b	1.4 ± 2.9 ab	0.6 ± 1.8 ab
KK 0.9	18.7 ± 20.6 a	4.3 ± 4.3 a	3.4 ± 3.4 a	3.2 ± 3.2 a	4.4 ± 4.6 a	3.4 ± 4.5 a	0.0 ± 0.0 a	0.0 ± 0.0 a
K	77.7 ± 10.1 c	15.7 ± 1.9 c	14.7 ± 3.0 d	13.9 ± 3.1 c	13.4 ± 3.4 c	10.4 ± 1.8 b	8.8 ± 3.4 c	2.0 ± 4.2 b

* Numbers followed by different quality letters in the same column indicate significantly different results (DMRT α=0.05)

Table 4. Mean multiplication rate and intrinsic growth rate of aphids in second generation

Treatment	Multiplication Rate (MR)	Intrinsic Growth Rate (rm)
KM 0.1	4.575 ± 0.571 b	0.089 ± 0.014 b
KM 0.3	4.132 ± 0.438 b	0.089 ± 0.011 b
KM 0.5	3.996 ± 0.829 b	0.098 ± 0.013 b
KM 0.7	4.164 ± 0.414 b	0.098 ± 0.014 b
KM 0.9	4.155 ± 0.553 b	0.088 ± 0.013 b
KM 1.1	4.388 ± 1.293 b	0.100 ± 0.014 b
KK 0.9	1.600 ± 1.743 a	0.048 ± 0.051 a
K	6.035 ± 0.752 c	0.106 ± 0.012 b

* Numbers followed by different quality letters in the same column indicate significantly different results (DMRT α=0.05)

This results in decreased insect fertility and fecundity, developmental delays, reduced size and weight, malformations, abnormal behavior or death (Lopez-Castillo et al., 2018). Antibiosis in insects can be evaluated from high mortality, low pest reproduction rates, and decreased reproductive ability of imago (Niks et al., 2011). The effect of antibiosis has also been reported to be able to reduce the fecundity of *A. glycines* in tolerant soybean plants (Hesler et al., 2007). In the first generation of aphids, chitosan treatment influenced the duration of the 2nd and 3rd instar nymph stages. The 2nd and 3rd instar nymphs in the KM 0.3 and KK 0.9 treatments, as well as the 3rd instar nymphs in the KM 0.9 treatment, exhibited longer nymph stages, with significant differences compared to other treatments and the control. However, chitosan treatment did not significantly affect the duration of the 1st and 4th instar nymph stages.

Pre-viviparity in chitosan-treated aphids did not show a significant effect. The life cycle of first-generation aphids ranged from 4.5 to 7.8 days, with the longest cycle observed in the KK 0.9 treatment (Table 1). The prolonged life cycle of aphids influences their reproduction rate, increasing the opportunity for natural enemies to prey on them (Listihani, 2015). The number of first nymphs born by the first generation of aphid imago in the KK 0.9 treatment was significantly lower when compared to other treatments and the control. The second generation of 1st instar nymphs in the KK 0.9 treatment, 3rd instar nymphs in the KM 0.9 treatment, and 4th instar nymphs in the KM 0.3 treatment showed longer nymph stages and were significantly different when compared to other treatments and the control. Chitosan treatment did not affect the pre-viviparity and life cycle of second generation aphids. The KK 0.9 treatment showed a shorter longevity and low fecundity (Table 2)

The decrease in aphid reproductive ability in KK 0.9 may be due to the fact that plants sprayed with chitosan were unable to meet their nutritional needs. Chitosan treatment also affected the total and daily characteristics of the second filial generation of aphid imagoe compared to the control (Table 3). The KK 0.9 treatment exhibited the lowest number of aphids on treated plants during the reproductive period, with daily frequencies ranging from 0 to 5.8 individuals per day. According to Kuswanto and Budi (2007), a decrease in the number of nymphs produced by each aphid imago can reduce its population. The presence of aphids on long bean plants treated with PGPR applications decreases because the absorbed protein is insufficient to meet their nutritional needs (Listihani, 2015). Feed quality plays a crucial role in feeding behavior and nutrient utilization. Poor feed quality leads to increased consumption, which in turn extends developmental time (Chown and Nicolson, 2004).

The multiplication rate (MR) and the intrinsic growth rate (rm) are constants used to describe the developmental dynamics of a population. These parameters are used to assess the suitability of habitat and food for insect growth and development. The higher the MR and rm values, the more suitable the habitat and food are for the insect (Laamari et al., 2008). The rate of aphid multiplication in all treatments showed lower values and was significantly different from the control. The intrinsic growth rate of aphids in the KK 0.9 treatment had the lowest intrinsic growth rate value. The lowest multiplication rate and intrinsic growth rate values were shown by the KK 0.9 treatment (Table 4). The KK 0.9 treatment had the best antibiosis effect, as indicated by a short lifespan, low virulence,

lower multiplication rate, and low intrinsic growth rate. Long life cycles result in extended generation times, decreased population growth, and reduced multiplication and intrinsic growth rates of insects (Kingsolver, 2007).

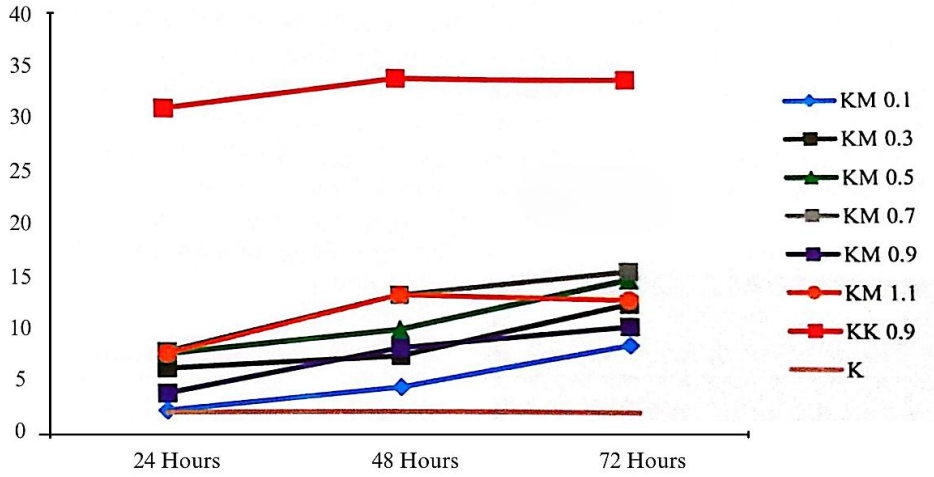

Figure 2. Mortality of aphids in chitosan treatment through direct spray method.

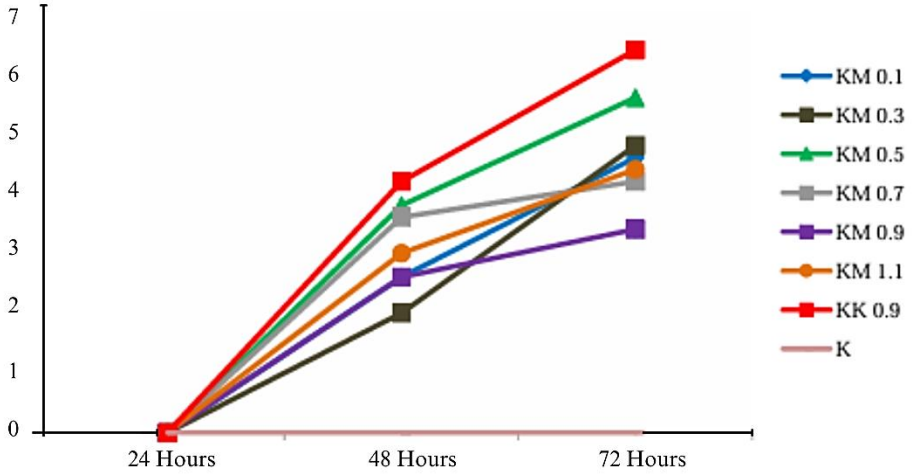

Figure 3. Mortality of aphids in chitosan treatment through systemic method.

Table 5. Toxicity effect of chitosan insecticidal activity on aphids

Treatment	Observation Time	b Value ± SE	LC_{50} (%)	LC_{95} (%)
Contact	24	3.20 ± 0.76	1.43	4.67
	48	2.15 ± 0.28	1.22	7.12
	72	0.99 ± 0.12	1.31	59.56
Systemic	24	-	-	-
	48	0.18 ± 0.14	0.17	-
	72	0.31 ± 0.12	-	-

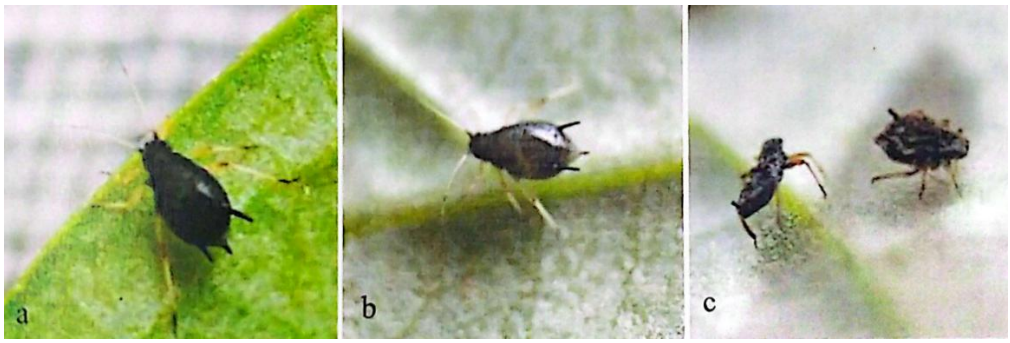

Figure 4. Physical characteristics of aphids due to chitosan treatment. a. healthy aphids b. naturally dead aphids c. aphids that have experienced lysis due to chitosan.

Insecticidal Activity of Chitosan Against Aphids

Pesticides are divided into two categories based on how they work: contact poisons and stomach poisons (systemic). Contact pesticides do not penetrate plant tissue or circulate within the plant's vascular system. Systemic pesticides, on the other hand, enter plant organs through the roots, stems, or leaves (Syarief and Hariadi, 1993).

Insecticides are substances containing toxic chemical compounds that can kill various types of insects. To kill insects, insecticides enter the insect's body through ingestion, contact, or respiration (Wudianto, 2007). The results of the chitosan insecticidal activity test, conducted using both the direct spray and residual methods, showed different outcomes in killing aphids. Greater and faster aphid mortality was observed in the direct spray method compared to the residual method. Aphid mortality in the direct spray method began 24 hours after treatment and continued to increase until 72 hours after treatment, ranging from 2% to 34.6%. The highest mortality was observed in the KK 0.9 treatment at 24, 48, and 72 hours after treatment (Figure 2).

Testing of chitosan insecticidal activity using the systemic method showed poor results in killing aphids. Aphid mortality in this method began only after 48 hours after treatment, with the highest mortality reaching just 6.4%, observed in the KK 0.9 treatment. The control treatment showed no aphid mortality (Figure 3). The effect of chitosan insecticidal activity through the direct spray method at 24 hours after treatment showed LC50 and LC95 values of 1.43% and 4.67%, respectively. These concentrations are higher than those used in this test. The insecticidal activity of chitosan through the systemic method at 24 JSP could not be calculated, possibly because the required concentration is too high (Table 5). Based on the results, chitosan is not effective in causing aphid mortality at the tested concentration; however, higher concentrations could cause toxicity to plants. Additionally, high chitosan concentrations are less economical when applied in the field.

The mortality of aphids caused by the insecticidal activity of chitosan is shown in Figure 4. Aphids that died due to chitosan treatment exhibited lysis and shriveling. Chitosan has been shown to increase the activity of the chitinase enzyme in inoculated plants (Meng et al., 2010). In insects, exposure to chitinase can cause significant structural changes in the peritrophic matrix, including peeling of the superficial layer,

rupture of the matrix, separation of the fibril network, and overall weakening of the tissue itself (Berini et al., 2019). These dead aphids are typically found under the leaf surface, where they remain attached. This result differs from the study by Lehane et al. (1997), which stated that aphid mortality due to chitosan insecticidal activity is caused by aphids being unable to molt. The insecticidal activity of chitosan is influenced by factors such as molecular weight and the presence of additional micro/macro elements (Badawy and El-Aswad, 2012).

CONCLUSION

Chitosan treatment effectively reduced aphid colonization and feeding preferences, and inhibited infestation. Additionally, chitosan treatment significantly decreased aphid reproduction, extended the life cycle, and reduced the growth rate. The direct spray method proved to be more effective in controlling aphid populations, whereas the systemic method showed reduced efficacy. The KK 0.9 treatment yielded the most favorable results across all parameters and time points.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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