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Evaluation of *Bacillus thuringiensis*-Based Bioinsecticide on The Presence of Arthropods in Vegetative Phase of Carrot

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Abstract

Synthetic insecticides are still extensively used by farmers to control insect pests in carrots. The effects of excessive use of synthetic insecticides can damage agricultural ecosystems. This research aimed to examine Bacillus thuringiensis-based bioinsecticide toward arthropods existence in vegetative growth phase of carrots The research was conducted in Pagar Alam City (700 m above sea level) and a relative humidity of 48–99%. The carrot plantations studied were planted in polyculture with mustard greens and sweet corn. This study used a completely randomized design (CRD) with 3 treatments and 9 replications. The treatments were bioinsecticide B. thuringiensis; synthetic insecticide (imidacloprid 200 g/l); and no-insecticide application (control). Agronomic observations were height of carrot and their number of leaves. Arthropods observations were carried out using sweep nets, pitfall traps, and direct visual observation. The results showed there was no significantly different on height of carrot plant and their number of leaves among three applications. Arthropods population in carrot plants treated with B. thuringiensis was lower than those in control carrot plants. In pitfall trap observations, the highest number of arthropod individuals obtained was belong to order Hymenoptera and had a moderate value of the Shannon-Wiener diversity index (H'). In addition, total insect population after application of B. thuringiensis observed using nets, tended to decrease from the second observation onwards. The category of insect diversity level trapped by Pitfall trap in B. thuringiensis bioinsecticide treatment was included in the medium category (H' = 1.75), while the treatment of imidacloprid (H' = 0.85) and control (H' = 0.81) was included in the low category.

Keywords : Bacillus thuringiensis, Bioinsecticide, Entomopathogenic, Plant growth, Daucus carota

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1. Introduction

Carrot (*Daucus carota* L.) is a horticultural commodity widely consumed by society because it has high nutritional value. Carrot consumption by the household sector increased by 7.35% or 26.90 thousand tons in 2021, and in 2022 it increased to 392.82 thousand tons [1]. The increase in demand must be balanced with high production. In reality, carrot cultivation is constrained by pest attacks. Pests of carrot crops were included *Agrotis* sp. earthworm, *Thysanoplusia orichalcea* caterpillar, *Myzus persicae*, *Cavariella aegopodii*, *Semiaphis heraclei*, *Aeolothrips meridionalis* [2] and *Melanagromyza* sp. larvae [3]. Farmers generally still use synthetic insecticides to control insect pests in their carrot crops. Excessive and continuous use of synthetic insecticides can cause resurgence of target pests and leave residues in cultivated plants and environment [4], [5]. In addition, synthetic insecticides can also kill natural enemies [6], [7].

The utilization of microorganisms such as *Bacillus* *thuringiensis* bacteria as biological agents is an alternative to reduce the use of synthetic pesticides. *B. thuringiensis* is a gram-positive bacterium that produces protein crystals during sporulation [8]. *B. thuringiensis* has been widely used in biological control of insect pests from different orders, including Lepidoptera, Coleoptera and Diptera [9]. The effectiveness of using *B. thuringiensis* has been reported in several cases such as in cabbage [10], caisim [11], other Brassicaceae families [12] and potato [13]. The use of *B. thuringiensis* does not have a negative impact on the environment. According to [14], application of bio-insecticide *B. thuringiensis* does not interfere with the abundance of predatory arthropods such as spiders and other predatory insects.

The application of *B. thuringiensis* in addition to being a bio-insecticide can also increase the growth of cultivated plants because it functions as a biofertilizer and biostimulator [15]. [16] also reported that *B. thuringiensis* can function as a bio-fertilizer in addition to its main role as a bio-insecticide. [17] emphasized that *B. thuringiensis* is a stimulant that has not been widely recognized by the public to increase plant growth. Therefore, research was conducted with the aim of studying *B. thuringiensis* bio-insecticide on carrot growth and the presence of arthropods in the vegetative phase of carrots.

2. Materials and Methods

The location of carrot cultivation in Keban Agung Village, Ulu Rurah Village, Pagar Alam Selatan Subdistrict, Pagar Alam City, South Sumatra Province, Indonesia (4º03'25 "S and 103º26'40") at an altitude of 700 m above sea level. The research design used a completely randomized design (CRD) with 3 treatments and 9 replicates. The treatments consisted of:

(1) Bio-insecticide *B. thuringiensis*
(2) Synthesized insecticide imidacloprid 200 g/l
(3) No application (water) as control.

2.1. Preparation of *Bacillus thuringiensis* bioinsecticide

The production of *B. thuringiensis* bio-insecticide was carried out in June 2023 at the Phytopathology Laboratory, Department of Plant Pests and Diseases, Plant Protection Study Program, Faculty of Agriculture, Sriwijaya University, Indralaya. *B. thuringiensis* isolate with isolate code BK originally from the bacterial collection at the Phytopathology Laboratory. Preparation of *B. thuringiensis* bio-insecticide using the method [18]. The colony density used was 5 x 10^6 CFU/ml.

2.2. Land preparation and carrot cultivation

Land preparation for making beds was done 2 weeks before planting. Beds were made measuring 10 m x 60 cm with a bed height of 50 cm. There were 27 units of beds made, with a distance of 20 cm between beds. Carrot planting was done by planting the seeds directly by making a furrow and then sowing the seeds in the furrow. Carrot seeds used were local variety. After the plants grew 45 days after planting (DAP), thinning was done with a spacing of 8 cm x 8 cm. Planting was done in polyculture by planting 5 rows of carrots in the center and 2 rows of mustard greens on the edge of the bed and interspersed with sweet corn plants..

2.3. *Bacillus thuringiensis* bioinsecticide application

The dose of *B. thuringiensis* used was 20 ml/L water. Bio-insecticide application was carried out by spraying on the plant crown and soil using an 8 L knapsack sprayer. Bio-insecticide application began when carrot plants were 3 weeks after planting (WAP). Applications were made 4 times with an application interval of 2 weeks. Applications were carried out every afternoon at 4-5 pm.

2.4 Test the effect of *Bacillus thuringiensis* bio-insecticide application on carrot vegetative growth

Observations of carrot vegetative growth including the number of leaves and plant height were carried out at the same time. The number of leaves and plant height were observed from 5 sample plants in each replication in treatment plots consisting of 9 replications. The number of leaves was counted then the plant height was measured using a crossbar after which the results obtained were recorded.

2.5 Observation of arthropods population

Observations were made visually, using insect nets and pitfall traps. Visual observations were made by looking directly at arthropods in treatment plots both before and after application of bio-insecticide *B. thuringiensis* and synthetic insecticides. Furthermore, the insects found were photographed and identified using the book [19] and browsing from the website http://www.bugguide.net/ managed by Iowa State University Entomology and PictureThis application. Population of insects observed was then counted and recorded. Observations using insect nets were made with 5 double swings. Observations using insect nets were conducted before and after application of *B. thuringiensis* bio-insecticide and synthetic insecticide. Observations using insect nets were made after visual observations. The insects obtained were then recorded and identified. Observations using pitfall traps were conducted every 2 weeks prior to bio-insecticide application, by setting one pitfall trap in each replicate and treatment. The method of pitfall trap installation was to make a hole in the ground 10 cm deep, then put a paralon pipe into the ground. Next, a clear plastic cup with a volume of 300 mL was inserted, then filled with soapy water (0.5%) as much as one-third the height of the plastic cup which was installed parallel to the ground surface to make it easier for insects to be trapped. The captured insects were then sorted, filtered with a 1 mm pore size sieve, rinsed with sterile water, then put into a jam bottle containing 70% alcohol, to be further identified under a microscope and counted the number of individuals in the Entomology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Sriwijaya University, Indralaya.

2.6 Data analysis

Regression analysis was performed to determine the relationship between parameters with coefficient determination (R2) indicating the strength of the relationship. Furthermore, significant differences between treatments were tested using the least significant difference (LSD) test at $P \leq 0.05$ by RStudio version 2023.06.2+561 "Mountain Hydrangea" Release for windows 10.

The data obtained from each insect capture were counted and identified, then analyzed using the Shannon-Wienner species diversity index formula [20] with the following equation: $H' = -\sum pi \ln pi$; $pi = ni / N$ (the ratio of the number of individuals of a species to the whole species), with ni: the number of individuals of the i-th species; N: total individuals. The species diversity index is defined as follows: $H' > 3$ indicates that species diversity is high, $1 \leq H' \leq 3$ indicates that species diversity is moderate, and $H' < 1$ indicates that species diversity is low.

3. Results and Discussion

3.1 Plant height (cm)

Based on the results of variance analysis, the application of *B. thuringiensis* had no significantly effect on plant height. However, the application of bio-insecticide *B. thuringiensis* had a positive effect in increasing plant height growth compared to the effect of other treatments. Plant height growth was faster from week 5 to week 9 and began to optimize when entering week 10 (Figure 1).

From the results of this study, it was found application of bio-insecticide *B. thuringiensis* had an effect in accelerating plant height. This was supported by results of research by [21] which states application of *B. thuringiensis* can increase the growth of chickpea plant height.

B. thuringiensis was considered to be able to increase plant growth because it has a function as a phytohormone which has an important function for plant growth and development as a regulator and signaler [22]. Phytohormone compounds are produced by bacteria colonizing plant roots that play a role in plant growth. Some strains of *B. thuringiensis* colonize plant roots and have properties that increase plant growth [23].

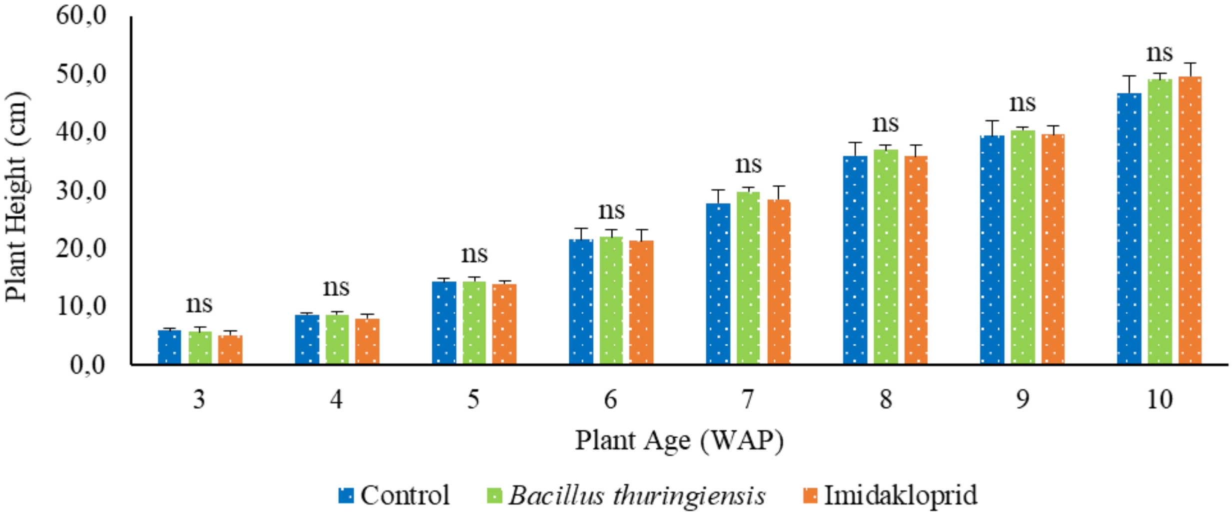

Figure 1. Effect of *Bacillus thuringiensis* bio-insecticide application on carrot plant height growth

3.2 Number of leaves (strands)

Based on the results of analysis of variance, application of *B. thuringiensis* caused no significant effect on the number of leaves. Based on the average application of *B. thuringiensis* obtained the highest number of leaves at the age of 5, 7 and 10 weeks after planting (Figure 2).

Based on the results of the study, the application of bio-insecticide *B. thuringiensis* was able to produce more leaves compared to other treatments. This is supported by the results of research by [24] which states *B. thuringiensis* treatment can increase the number of leaves on chili (*Capsicum annuum*) and long beans (*Vigna unguiculata*).

The increase in number of leaves in *B. thuringiensis* treatment was allegedly because *B. thuringiensis* can function as a bio-fertilizer because it was able to dissolve phosphate [25]. This is also supported by [17], stated that some Bacillus genera have the ability to fix N from the air, synthesize IAA to spur plant growth and suppress pathogens and dissolve P and K.

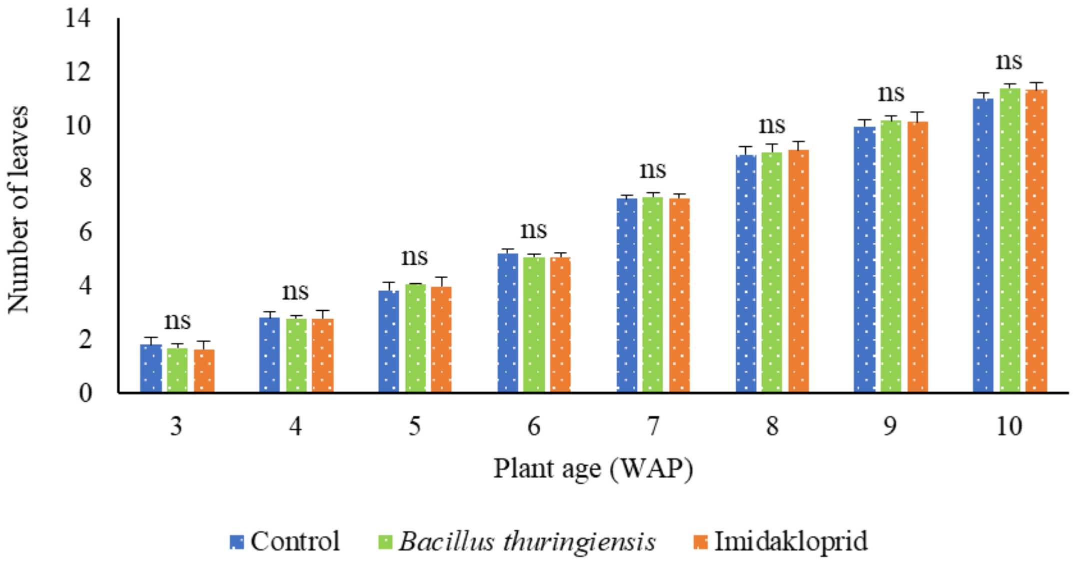

Figure 2. Effect of *Bacillus thuringiensis* bio-insecticide application on the growth of the number of leaves of carrot plants

3.3 Visual insect population

Visual insect population observations showed differences in the total insect population observed visually before and after the application of bioinsecticide *B. thuringiensis*, synthetic insecticide and control. The total insect population of *B. thuringiensis* treatment in the first observation decreased after application but increased in the 2nd, 3rd and 4th observations (Figure 3).

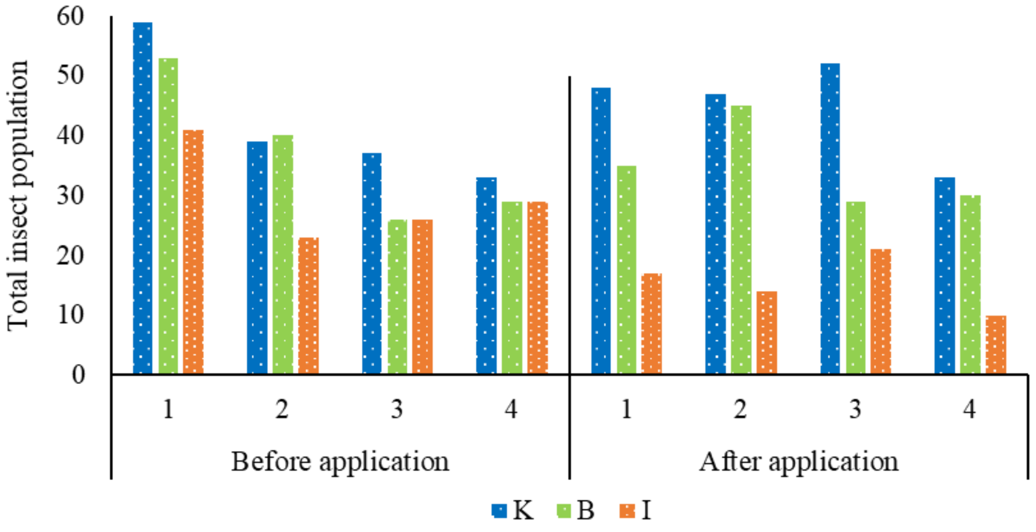

Figure 3. Total insect population before and after application of bio-insecticide *B. thuringiensis* (B), synthetic insecticide (P) and no application (K) observed visually.

Based on the results of study, effect of *B. thuringiensis* application on the presence of insects fluctuated. This was due to the use of bioinsecticide *B. thuringiensis* which has a slow effect in suppressing host-specific insects, in contrast to the application of synthetic pesticides that work quickly in killing insects [11].

3.4 Insect population with nets

The results showed that there were differences in the total insect population before and after application. The total insect population due to *B. thuringiensis* bio-insecticide treatment in the 2nd, 3rd and 4th observations decreased (Figure 4).

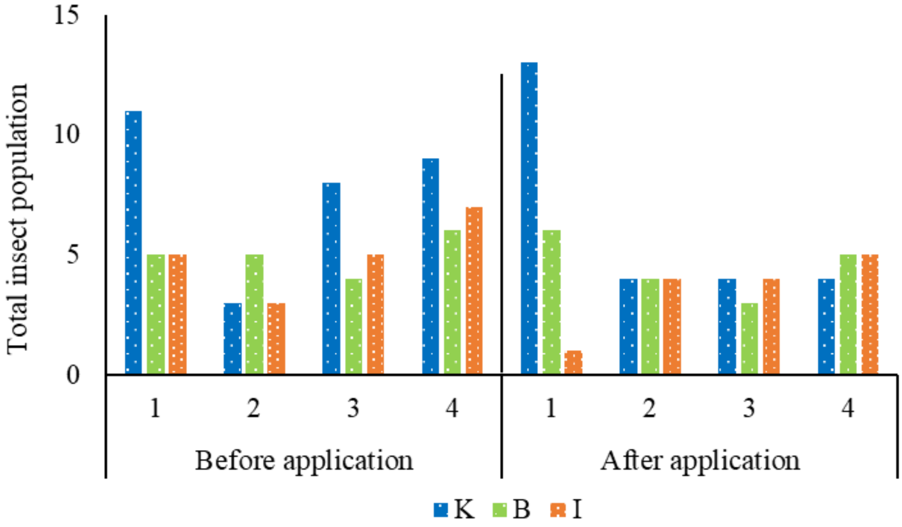

Figure 4. Total insect population before and after application of bioinsecticide *B. thuringiensis* (B), insecticide imidakloprid (I) and no application (K) observed by netting.

The decrease insect population in *B. thuringiensis* bio-insecticide treatment was alledly because *B. thuringiensis* possesses δ-endotoxin protein crystals that will damage the cell membrane structure in the host insect. It can weaken the immune system and kill the host insect after a few days of infection [8].

The number of insect populations in polyculture will decrease when the crop was harvested [26]. This is due to the various types of crops whose harvest times vary, causing insect emigration out of cultivated crops. The presence of natural enemies obtained in the observations can suppress insect pest populations. This is due to increased biodiversity caused by polyculture cropping patterns [27].

3.5 Arthropod population with pitfall trap

Based on the results of the study, the category of insect diversity level in the B. thuringiensis bio-insecticide treatment was moderate (H' = 1.75). The synthetic insecticide treatment (H' = 0.85) and control (H' = 0.81) were categorized as low insect diversity (Table 1).

The use of bio-insecticide *B. thuringiensis* did not cause the diversity of arthropod species low. Arthropod population density will change because it was influenced by biotic and abiotic factors. Biotic factors such as the presence of natural enemies and vegetation diversity and abiotic factors such as temperature played an important role in insect life [28], [29]. It is proven from this study that abiotic factors such as fluctuating humidity can affect the insect population around the plant. Relative humidity at the time of carrying out observations ranged from 48-99%.

In addition, the polyculture system in the field causes a more diverse insect population when compared to the monoculture system or only carrot crops.

Table 1. Arthropods species trapped by pitfall trap

Order/family	Species	Found in every treatment (individue)			Total (individue)
		Bt	I	K	
Coleoptera					
Staphylinidae	*Anotylus* sp.	1	2	2	5
Carabidae	*Pherosophus bimaculatus*	2	2	0	4
Coccinellidae	*Coccinellidae* sp.	0	0	2	2
Orthoptera					
Gryllidae	*Gryllus pennsylvanicus*	10	4	4	18
Diptera					
Muscidae	*Graphomya maculata*	1	0	0	1
Hymenoptera					
Formicidae	*Odontoponera denticulata*	42	36	34	112
	Tapinoma sessile	26	12	7	45
	Camponotus americanus	1	0	2	3
	Anoplolepis gracilipes	10	12	15	37
Polydesmida					
Paradoxosomatidae	*Asiomorpha coarctata*	6	7	5	18
Araneae					
Pisauridae	*Dolomedes plantarius*	14	17	9	40
Biodiversity index		1,75	0,85	0,81	
Dominancy index		0,23	0,03	0,02	
Evennes index		0,76	0,40	0,37	

Notes: Bt: *Bacillus thuringiensis*, I: Imidacloprid, K: control

3.6 Species of arthropods

Thirty-six species of arthropods were collected in carrot crops during vegetative phase by visual observation, pitfall traps and insect nets. These arthropods have roles as pests (Figure 7), natural enemies (Figure 8) and pollinators (Figure 9).

In the bioinsecticide *B. thuringiensis* treatment, the arthropod species that acted as pests were the most and those that acted as pollinators were the least compared to the imidacloprid and control treatments (Figure 10).

This is because *B. thuringiensis* in controlling insects that are not host specific so that the effect is not significant. Although in some studies, *B. thuringiensis* toxin has successfully controlled Coleoptera and Lepidoptera pest insects, Hemiptera pest insects are not very susceptible to *B. thuringiensis* toxin [30].

According to [31], little is known about the toxicity of *B. thuringiensis* to Hemiptera that have a sucking mouth type (haustelata).

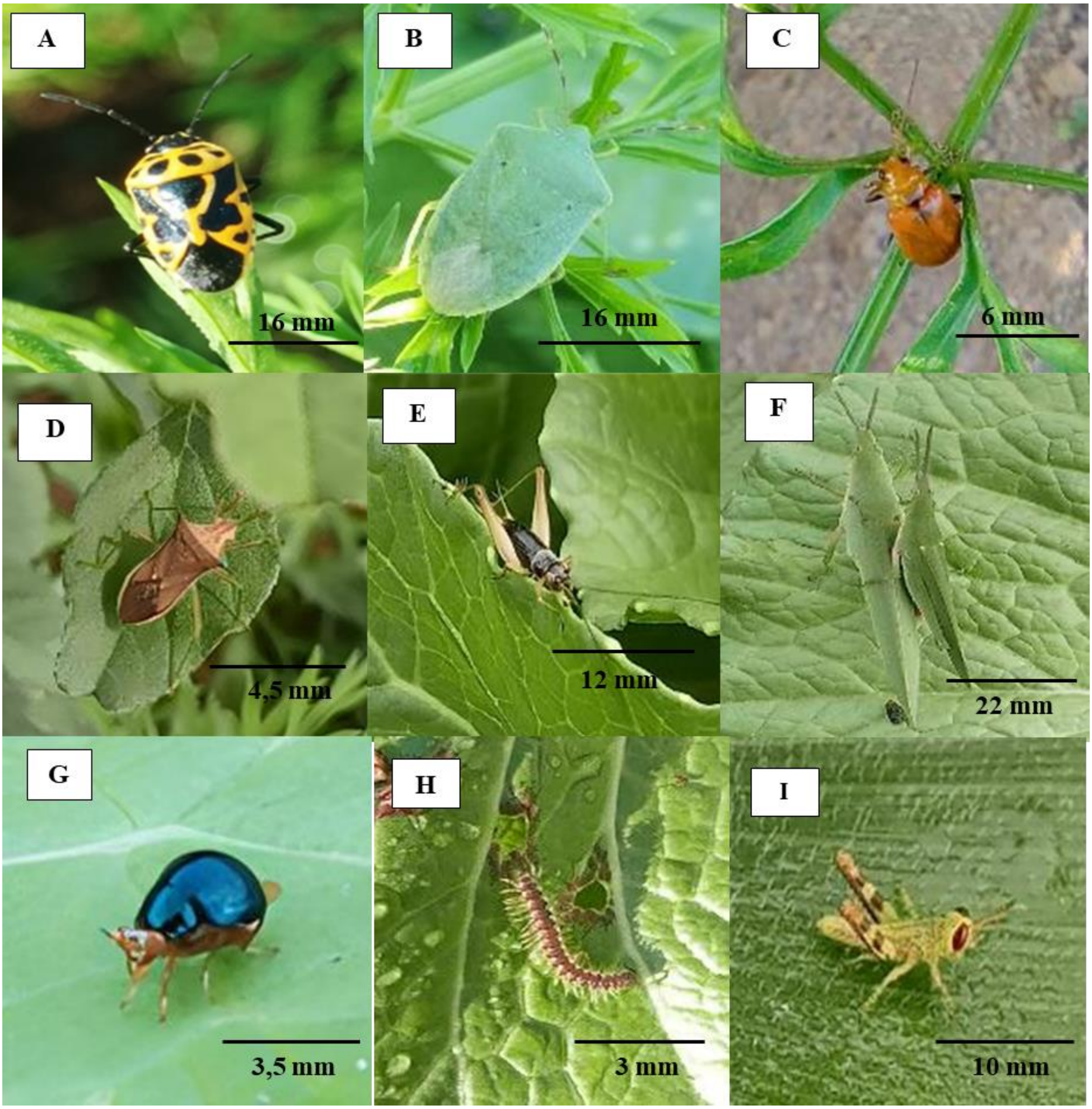

Figure 7. Species arthropods of pests: *Eurydema pulchrum* (A), *Nezara viridula* (B), *Aulocophora similis* (C), *Cletus schmidti* (D), *Phyllopalpus pulchellus* (E), *Acrida turita* (F), *Celyphus* sp. (G), *Asiomorpha coarctata* (H), *Patanga* sp. (I).

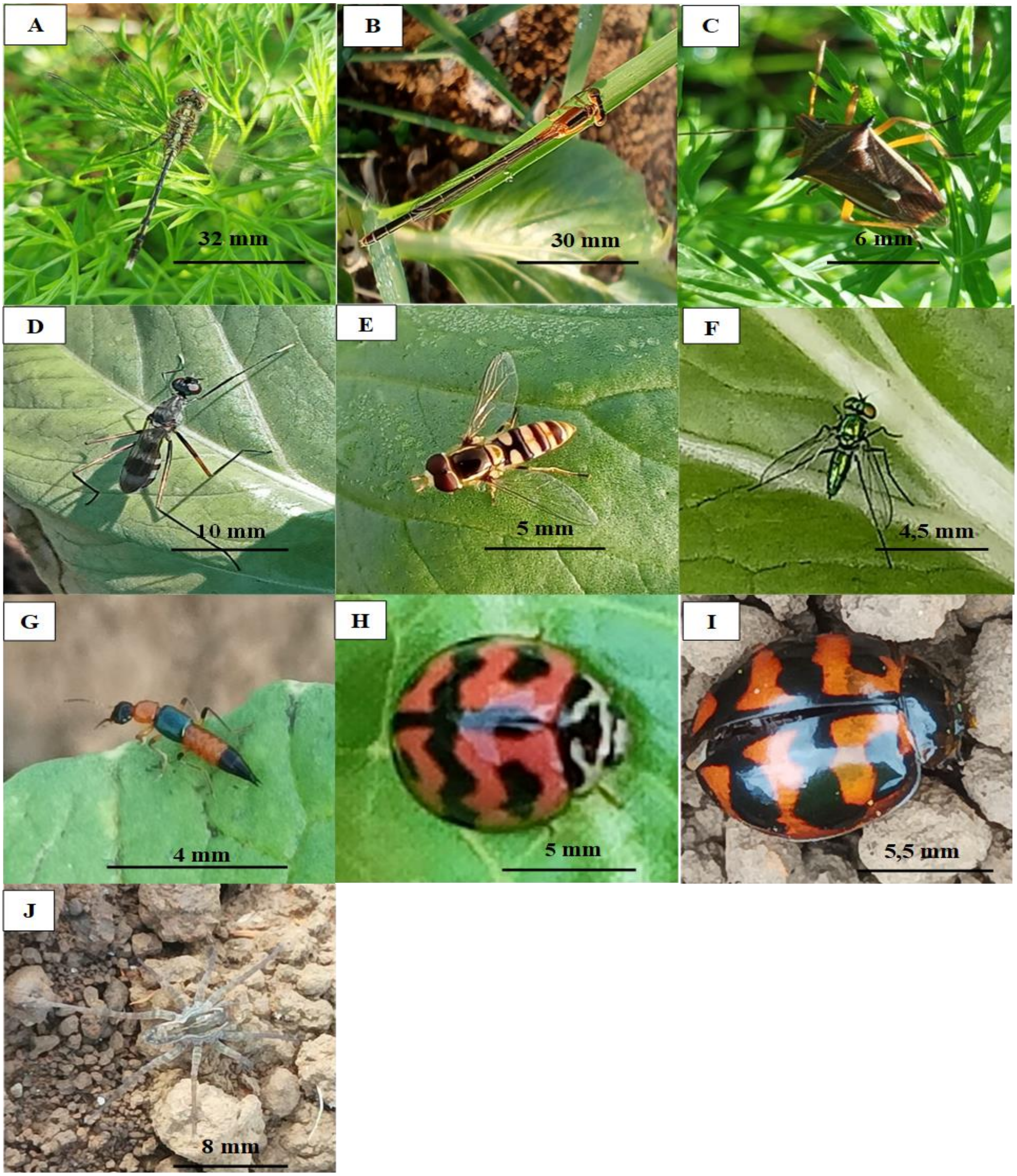

Figure 8. Species predatory arthropodes: *Diplacodes trivialis* (A), *Ischnura ramburii* (B), *Andrallus spinindens* (C), *Rainieria antennaepes* (D), *Ischiodon scutellaris* (E), *Condylostylus* sp. (F), *Paederus fuscipes* (G), *Menochilus sexmaculatus* (H), *Coccinela transversalis* (I), *Pardosa pseudoannulata* (J).

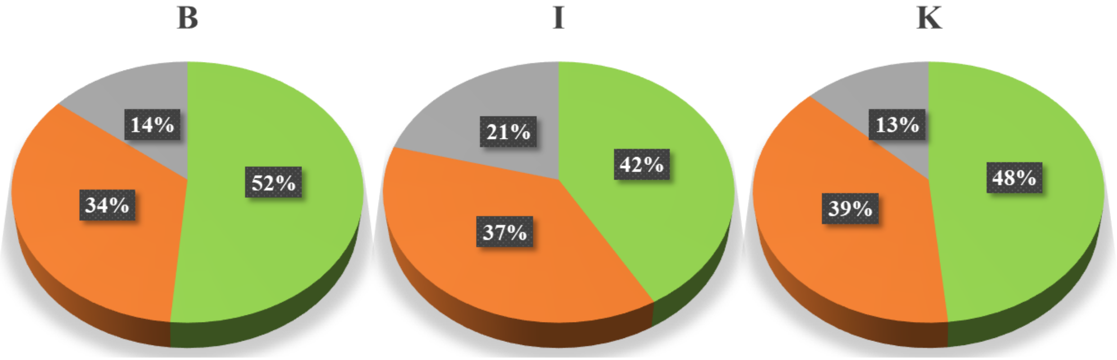

Figure 9. The role of arthropods in carrot crops during the vegetative phase
Notes: B: *Bacillus thuringiensis*, I: Imidacloprid, K: control, 🟩 Pests, 🟧 Natural enemies, ⬜ Pollinator

4. Conclusion

The application of bio-insecticide *B. thuringiensis* had no significant effect on the growth of height and number of carrot leaves. In *B. thuringiensis* bio-insecticide treatment, the arthropod species acted as pests was the highest and those acted as pollinators was the lowest compared to the imidacloprid and control treatments. The category of insect diversity level trapped by Pitfall trap in *B. thuringiensis* bio-insecticide treatment was included in the medium category (H' = 1.75), while imidacloprid treatment (H' = 0.85) and control (H' = 0.81) were low category.

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