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Response of Arthropod Diversity in Rice (*Oryza sativa* L.) to Application of Brotowali (*Tinospora crispa* L.) Stem Botanical Insecticide

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Abstract: Botanical insecticide from Brotowali (Tinospora crispa) stems offers an alternative pest control method that is safer for non-target organisms. This study aimed to identify the bioactive compounds of Brotowali stems, determine LC_{95} toxicity, and evaluate their effects on arthropod diversity and rice yield. Research was conducted at the Bioscience and TPB Laboratories of Politeknik Negeri Jember and in rice fields at Balung Lor Village, Jember. Arthropods were collected using sweep nets, sticky traps, yellow pan traps, and pitfall traps. Field trials compared plots treated with either fipronil or Brotowali bioinsecticide. GC-MS analysis revealed dominant compounds: hexadecanoic acid (17.40%), oleic acid (16.25%), and acetic acid (11.82%). LC_{95} toxicity was 40.178%. Arthropod observations recorded 2,090 individuals from 32 species in the Brotowali plot, versus 872 individuals from 27 species in the fipronil plot. Both treatments showed moderate diversity (H), no species dominance (C), moderate richness (R), even distribution (E), and high similarity (91.53%). Rice yield differed significantly (Mann-Whitney test). Overall, Brotowali stem bioinsecticide maintained arthropod diversity and improved rice yield, showing potential as an eco-friendly pest control strategy.

Keywords: Botanical insecticide, Arthropods

1. Introduction

Rice is the main food commodity in Indonesia, with most of the population relying on it as the primary source of carbohydrates. The increasing population is directly proportional to rice demand, while agricultural land availability continues to decline due to land conversion. In 2021, the harvested rice area decreased by 2.3% or about 245.7 thousand hectares, with a total production of 54.42 million tons of dry milled grain, a 0.43% decline compared to the previous year [1]. This condition requires farmers to maximize productivity through proper cultivation management.

One crucial aspect of rice cultivation is the presence of arthropods, which act as pests, natural enemies, or neutral organisms [2,3]. The balance of the paddy field ecosystem can be reflected in the diversity of arthropod populations. However, farmers commonly rely on synthetic pesticides for pest control. Continuous use of chemical pesticides not only kills target pests but also reduces populations of natural predators and pollinators, increases pest resistance, and contaminates soil and water. These impacts may decrease soil fertility, disrupt ecosystem balance, and threaten the health of farmers and surrounding communities [4].

A more environmentally friendly alternative is the use of botanical insecticides, such as those derived from Brotowali (Tinospora crispa) stems. Brotowali stems contain bioactive compounds, including alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids, which exhibit toxic and antifeedant effects on insects [5]. Therefore, research on the effectiveness of Brotowali stem insecticides and their impact on arthropod diversity in rice fields is necessary to be carried out.

2. Materials and Methods

This study consisted of two stages. The first stage was conducted in the Plant Protection Laboratory, and the second stage was carried out in the rice fields of Balung Lor Village, Balung District, Jember, East Java.

2.1. Tools and Materials

The tools used included gauze, small transparent jars, rubber bands, measuring cylinders, knives, containers, cutting boards, blender, tweezers, digital balance, writing tools, raffia string, bamboo, knapsack sprayer, camera, and measuring tape. Materials included Inpari 32 rice plants, rice panicles at the milk stage, Leptocorisa oratorius (rice bug), distilled water, synthetic insecticide, brotowali stem extract, and observation sheets.

Mortality and Toxicity Test

Six concentrations of brotowali stem extract were tested with three replications:

P1 = Control (distilled water)	P4 = 15% (75 ml extract + 425 ml distilled water)
P2 = 5% (25 ml extract + 475 ml distilled water)	P5 = 20% (100 ml extract + 400 ml distilled water)
P3 = 10% (50 ml extract + 450 ml distilled water)	P6 = 25% (125 ml extract + 375 ml distilled water)

Rice panicles were dipped into each treatment solution, air-dried, and placed in transparent jars containing five rice bugs, covered with gauze secured by rubber bands. Mortality was observed at 24, 48, 72, and 96 hours after application. Toxicity levels (LC_{50} and LC_{95}) were determined using Probit analysis with PoloPlus 1.0 software.

Preparation of Botanical Insecticide: A total of 500 g of brotowali stems were washed, chopped, and blended with 500 ml of water (1:1 ratio). The mixture was left for 24 hours, filtered, and stored in a measuring cylinder. Field Experiment: The field study evaluated the effectiveness of brotowali extract against arthropod diversity. Two plots were prepared: Plot 1 sprayed with synthetic insecticide containing fipronil; Plot 2 sprayed with brotowali extract (effective concentration from laboratory test).

Spraying was performed weekly in the morning using a knapsack sprayer, starting from the milk stage (±70 days after transplanting). Arthropods were monitored visually and with insect traps, including sticky traps, yellow pan traps, pitfall traps, and sweep nets.

Trap Installation: Three types of traps were used in the field: sticky traps (bright colors), pan traps, and pitfall traps. Additionally, manual sweep net sampling was conducted. Observation and Sampling of Arthropods. Observations were carried out weekly at 6, 7, 8, and 9 weeks after transplanting (WAT), between 06:00–09:00 a.m. on clear weather days. Arthropods were recorded visually within designated plots and collected using traps. Data were classified by order, family, species, and number of individuals per species. Arthropod Identification: Collected arthropods were preserved and identified under a microscope at the Seed Production Technology Laboratory, Politeknik Negeri Jember. The number of species was recorded for each sampling event and classified into orders, families, and species. Data analysis included calculation of

Shannon–Wiener diversity index, Simpson dominance index, Margalef richness index, evenness index, and Sorensen similarity index.

3. Results and Discussion

Based on the observation data of the study on the effectiveness of the botanical insecticide from brotowali stems (Tinospora crispa L.) on the diversity of arthropods in rice plants (Oryza sativa), the results are as follows :

3.1. Results of GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

GC-MS is an organic compound separation technique that combines two analytical methods: gas chromatography (GC) for quantitative determination of compound amounts and mass spectrometry (MS) for identification of the molecular structures of the analyzed compounds [6]. Based on the analysis of the botanical insecticide from brotowali stems conducted by the Bioscience Laboratory of Jember State Polytechnic using a GC-MS instrument, a total of 37 constituent compounds were identified.

Table 1. Mass spectrum of botanical insecticide from brotowali stem.

No. Peak	Retention time	Compound Name	Concentration (%)
1	2.300	Peracetic Acid	1.13
2	3.000	Ethanamine, N-methyl-	1.88
3	3.171	N-Vinylaziridine	2.16
4	3.280	Acetic acid (CAS) Ethylic acid	11.8
5	3.649	2-Butenal, 4-(acetyloxy)-	3.15
6	3.821	Ethanol, 2-(dimethylamino)- (CAS) N, N-Dimethylethanolamine	1.79
7	4.220	Butane, 2-ethoxy- (CAS) Ethyl sec-butyl ether	0.83
8	4.757	2,3-Butanediol (CAS) Butane-2,3-diol	1.29
9	15.176	4-Methyl-5h-Furan-2-One	0.27
10	17.753	Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-(CAS) 1-Acetyl-2-methylcyclopentene	0.20
11	18.390	Methyl-2-Furoate	0.50
12	19.211	Ethylcyclopentenolone	0.23
13	20.651	2-Pyrrolidinone (CAS) Pyrrolidone	1.76
14	21.521	Hexanoic acid, 2-propenyl ester (CAS) Allyl caproate	0.36
15	23.318	Isosorbid	0.92
16	24.454	1-Octanol, 2-butyl- (CAS) 2-Butyl-1-octanol	0.51
17	24.866	5-Hydroxymethyl-Dihydro-Furan-2-One	0.55
18	25.962	Decanoic acid (CAS) Capric acid	0.85
19	26.918	Cyclododecane (CAS)	0.37
20	28.553	1,4-Benzenediol (CAS) Hydroquinone	0.13
21	29.213	1-Nonadecene (CAS)	0.46
22	30.455	Dodecanamide, N,N-bis(2-hydroxyethyl)-	0.35
23	31.327	1-Nonadecene (CAS)	0.29
24	31.529	cis-anti-cis-Tricyclo[7.3.0.0(2,6)]dodecan-7-o	0.27
25	31.966	Octadecanoic acid (CAS) Stearic acid	0.28
26	33.621	Hexadecanal (CAS) Palmitic Aldehyde	0.25
27	34.538	Tetradecanoic acid (CAS) Myristic acid	2.69
28	35.523	Pentadecanoic acid, 14-methyl-, methyl ester (CAS) Methyl 14-Methyl-Pentadecanoate	1.41
29	36.123	Tetradecanenitrile (CAS) Tetradecanonitrile	0.27
30	37.404	1-Eicosanol (CAS) n-Eicosanol	0.44
31	39.332	Hexadecanoic acid (CAS) Palmitic acid	17.4
32	42.697	9-Octadecenoic acid (Z)- (CAS) Oleic acid BUTANAL, (S)-2-	16.2
33	43.622	[(Tert.Butyloxycarbonyl)Amino]-3-Methyl-	9.42
34	45.908	Cyclododecene, (E)- (CAS) (E)-Cyclododecene	7.26
35	46.706	10-Dodecen-1-AL	8.25

36	50.073	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester (CAS) 1-Palmityl-2,3-Diacetin	2.99
37	53.460	Cyclopentadecanone, 2-hydroxy-	1.03
		Jumlah	100.00

Compounds with high percentages include Hexadecanoic acid (CAS), Palmitic acid (17.40%); 9-Octadecenoic acid (Z)- (CAS) Oleic acid (16.25%); and Acetic acid (CAS) Ethylic acid (11.82%). The botanical insecticide from brotowali stems contains a high concentration of acetic acid. Upon direct contact with pests, acetic acid can cause mortality by damaging the permeability of the insect cuticle. Its effectiveness is higher through direct contact than indirect exposure [7].

3.2. Toxicity of Botanical Insecticide from Brotowali Stems

The LC95 toxicity test of the botanical insecticide from brotowali stems showed a lethal concentration against test insects at 40.178% with a confidence interval of 22.246–75.849%. The use of LC95 is applied because field spraying requires a dosage capable of killing up to 95% of the target pest population. This is due to the correlation between exposure time and concentration, as pests in the field are exposed to the botanical insecticide extract for only a short period. Therefore, a relatively high concentration is needed to achieve a high mortality rate. [8].

3.3. Arthropod Diversity

Table 2. Observations of arthropods in rice cultivation fields located in Balung Lor Village, Balung Subdistrict, Jember Regency

No	Spesies	Ordo	Family	Role	Treatment	
					Botanical Insecticide Brotowali stem	Fipronil
1	*Nilaparvata lugens*	Hemiptera	Delphacidae	Herbivor	+	+
2	*Scirpophaga innotata*	Lepidoptera	Crambidae	Herbivor	+	+
3	*Conocephalus longipennis*	Orthoptera	Tettigonioidea	Herbivor	+	+
4	*Cnaphalocrosis medinalis*	Lepidoptera	Crambidae	Herbivor	+	+
5	*Leptocorisa oratorius*	Hemiptera	Alydidae	Herbivor	++	++
6	*Valanga nigricornis*	Orthoptera	Acrididae	Herbivor	+	+
7	*Oxya chinensis*	Orthoptera	Acrididae	Herbivor	++	+
8	*Chilo suppresissialis*	Lepidoptera	Crambidae	Herbivor	+	+
9	*Metioche vittaticollis*	Orthoptera	Gryllidae	Herbivor	+	+
10	*atractomorpha crenulate*	Orthoptera	Pyrgomorphidae	Herbivor	+	+
11	*Mantis religiosa*	Mantodea	Mantidae	Predator	+	-
12	*Orthetrum sabina*	Odonata	Libellulidae	Predator	+	+
13	*Agriocnemis femina*	Odonata	Coenagrionidae	Predator	+	-
14	*Iridomyrmex purpureus*	Hymenoptera	Formicidae	Predator	++	++
15	*pheropsophus jessoensis*	Coleoptera	Carabidae	Predator	+	+
16	*Ophionea nigrofasciata*	Coleoptera	Carabidae	Predator	+	+
17	*Paederus littoralis*	Coleoptera	Staphylinidae	Predator	++	+
18	*Coccinella transversalis*	Coleoptera	Coccinellidae	Predator	++	++
19	*Oxyopes javanus*	Araneae	Oxyopidae	Predator	+	-
20	*Lycosa pseudoannulata*	Araneae	Lycosidae	Predator	+	+
21	*Ardistomis seriepunctatus*	Coleoptera	Carabidae	Predator	+	+
22	*Verania lineata*	Coleoptera	Coccinellidae	Predator	+	+
23	*sciapus platypterus*	Diptera	Dolichopodidae	Parasitoid	++	+
24	*sepedon sphegea*	Diptera	Sciomyzidae	Parasitoid	+	+
25	*Pipunculus mutillatus*	Diptera	Pipunculidae	Parasitoid	+	+
26	*Trigoniulus corallinus*	Spirobolida	Trigoniulidae	Detrivor	+	-
27	*Musca domestica*	Diptera	Muscidae	Detrivor	+	+
28	*Amara aenea*	Coleoptera	Carabidae	Detrivor	+	+
29	*Melanitis leda*	Lepidoptera	Nymphalidae	Polinator	+	+
30	*megachile relativa*	Hymenoptera	Megachilidae	Polinator	+	-

31	*Junonia hedonia*	Lepidoptera	Nymphalidae	Polinator	+	+
32	*Mycalesis perseus*	Lepidoptera	Nymphalidae	Polinator	+	+

[1] (+) there are species, (-) there are no species, (++) number of species >100, (+) number of species <50.

Table 2 presents the results of both treatments. Application of the botanical insecticide from brotowali stems recorded a total of 32 species, consisting of 10 herbivores, 12 predators, 3 parasitoids, 3 detritivores, and 4 pollinators, with a total of 2,090 individuals. In contrast, the use of synthetic insecticide with the active ingredient fipronil recorded 27 species, consisting of 10 herbivores, 9 predators, 3 parasitoids, 2 detritivores, and 3 pollinators, with a total of 872 individuals.

3.4. Arthropod Diversity Index

Indeks	Perlakuan	
	Botanical insecticide from brotowali stems.	Fipronil
Shannon-Wiener Diversity Index (H')	2,8	2,7
Simpson's Dominance Index (C)	0,08	0,09
Margalef Species Richness Index (R)	4,05	3,98
Species Evenness Index (E)	0,8	0,8
Sorensen Species Similarity Index (ISS)	91,53%	91,53%

The data obtained from fields treated with botanical and synthetic insecticides fall into the medium Shannon-Wiener diversity index category because the value of (H') < 3.22 [9]. The dominance index (C) in both fields was 0.08 for the plot treated with the botanical insecticide from brotowali stems, and 0.09 for the plot treated with the synthetic insecticide fipronil. The dominance index indicates whether a certain species dominates the ecosystem in the area. The Margalef species richness index in the field treated with the botanical insecticide from brotowali stems was 4.05, while the field treated with the synthetic insecticide recorded 3.98. Since the value of (R) is > 3.5 and < 5, species richness is classified as medium [10]. The evenness index values obtained from both fields were 0.8% each. Species evenness (E) ranges from 0 to 1, and the closer the value is to 1, the more evenly distributed the species. The Sorensen similarity index value between the two fields was 91.53%, indicating that the species composition in both fields with different insecticide applications remains largely similar.

4. Conclusions

The GC-MS analysis of the botanical insecticide from brotowali stems identified 37 types of compounds, with the highest concentration compounds being Hexadecanoic acid (CAS) Palmitic acid (17.40%), 9-Octadecenoic acid (Z)- (CAS) Oleic acid (16.25%), and Acetic acid (CAS) Ethylic acid (11.82%), which have potential as botanical insecticides. The LC95 value of the brotowali stem botanical insecticide, used as a reference for field spraying, was 40.178%. The treatment with the botanical insecticide recorded 32 species with a total of 2,090 individuals, while the application of Fipronil recorded 27 species with a total of 872 individuals. The Shannon-Wiener diversity index (H') in both treatments indicated a medium level of arthropod diversity. The Dominance Index (C) showed no dominance of a single species in either treatment. The species richness index (R) was classified as medium, the evenness index (E) indicated evenly distributed species in both treatments, and the species similarity index (ISS) showed similarity between the two.

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