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(RESEARCH ARTICLE)

Synergistic and Sublethal Effects of Agrochemical Mixtures on *Porcellio laevis*: A Chronic and Acute Toxicity Assessment

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Abstract

Contamination of terrestrial habitats by pesticides presents an ecological issue of significance due to an impact on non-target terrestrial invertebrates. The terrestrial isopod, *Porcellio laevis*, as a cosmopolitan species, is significant as a bioindicator due to its functions in decay and nutrient cycling. Since lethal endpoints continue to be of the most significance in most studies, sublethal impacts in relation to changes in growth, breeding, and behavior offer earlier and ecologically relevant indicators of distress. This study estimated acute and chronic toxic impacts of common pesticides, Cyperkill (an insecticide), Garlon (an herbicide), and Benlate (a fungicide), individually and in combination in binomial mixtures. Adult *Porcellio laevis* were treated with treated leaves and soil amended with Cyperkill (0.15, 0.30, 0.60 ppm), Garlon and Benlate (300, 600, 900 ppm), and three binary mixtures (Cyperkill + Garlon, Cyperkill + Benlate, and Garlon + Benlate) for 8 weeks. Changes in body weight, food consumption, juvenile production, and rates of mortality. LC_{50} of Cyperkill was estimated in an acute toxicological bioassay after 24 h and 48 h through Probit regression analysis. The juvenile output and body weight dropped considerably under all pest control treatments, most notably under multiple exposures, indicating evidence of synergistic toxicity. Food consumption was not significantly different under treatment, however, there was an overall trend of a drop under pest control treatment. Percentage mortality increased with an increase in the concentration of pest control, and maximum lethality was caused by the treatment combination of Cyperkill + Garlon. The LC_{50} value of Cyperkill decreased from 24 h to 48 h, indicating increased toxicity after prolonged exposure durations. These findings confirmed that both single and mixed exposures to pesticides have adverse effects on the main physiological and reproductive indices of *Porcellio laevis*. The combination of sublethal criteria, in correlation with mortality indicators, provides a more comprehensive characterization of ecological risk. This validates the significance of mixture research and behavior-based biomarkers in ecotoxicological assessments of soils.

Keywords: *Porcellio laevis*; Agrochemical mixtures; Sublethal toxicity; Synergistic effects; Ecotoxicology

1. Introduction

Pesticide contamination of terrestrial ecosystem soils poses a serious threat to non-target invertebrates [1-3], which carry out vital ecosystem functions such as litter decomposition [4] as well as nutrient cycling. Of these, the terrestrially living isopods such as *Porcellio laevis* are found to be good bioindicators as they exhibit sensitivity towards ecological pollutants and play an important role in litter detritivore ecology [5,6,8].

In agriculture, these organisms receive chronic exposures to sub-lethal doses of a range of pesticides through treated leaves as well as polluted soil [9,10]. These pesticides are rarely used separately; instead, exposures almost always encompass multi-component mixtures [11], administered singly or accumulated through repeated use. Thus,

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toxicologic investigations that address mixture effects [12], as opposed to a single chemical entity, are required for realistic exposure risk analyses in the field [13,14].

This study assesses the chronic and acute effects of three common commercial pesticides, Cyperkill (a cypermethrin-containing insecticide), Garlon (a triclopyr-containing herbicide), and Benlate (a benomyl-containing fungicide), when used separately and in combination, on *Porcellio laevis*. In contrast to synthetic agrochemicals, natural products have also been studied for their biological activity as alternatives for more environmentally friendly management tactics against pests [15]. These pesticides belong to three significant classes: pyrethroids, carbamates, and organochlorine analogs, with various modes of action that can potentially result in synergistic or antagonistic effects when co-applied [5,13,16,17]. Cypermethrin is a potent neurotoxin that affects voltage-gated sodium channels. Benomyl has been found to impair mitosis [18], and Garlon affects auxin regulation in plants that could cause undesirable off-target effects on invertebrates [19-21].

Traditional toxicological endpoints tend to rely on lethality (e.g., LC_{50}). However, increasing evidence suggests that sublethal endpoints such as changes in growth, feeding, and reproduction can be more sensitive ecological indicators of recognizing long-term toxicity [5,22-24] In addition, toxic thresholds such as LC_{50} as well as IC_{50} values can vary based on exposure duration as well as recovery time as a result, highlighting the significance of time-dependent studies in ecotoxicological investigations [25]. LC_{50} value determination has traditionally depended on Probit analysis first standardized by [26], as this analysis remains the standard method for measuring pesticide toxicity in ecotoxicological studies. Thus, this study explores a range of diverse endpoints, including body weight change, food consumption, juvenile production, mortality and acute toxicity, to provide a thorough evaluation of both sublethal and lethal effects.

By assessing responses to both chronic low-dose exposure and acute toxicity tests, we aim to establish a more comprehensive toxicological profile of these pesticides and their mixtures. This study underscores the importance of incorporating mixture toxicity, behavioral, and reproductive endpoints in terrestrial ecotoxicological studies, thereby advancing our understanding of how soil detritivores, such as *Porcellio laevis*, are affected by pesticide contamination.

2. Materials and Methods

2.1. Test Organism

Adult terrestrial isopods (*Porcellio laevis*), a common soil-dwelling detritivore, were collected from pesticide-free compost areas in eastern Libya and acclimatized under laboratory conditions for two weeks before experimentation. The organisms were kept in plastic containers lined with moistened, untreated soil and leaf litter and maintained under controlled conditions (22 ± 2 °C, 70–80% relative humidity, 12:12 light:dark photoperiod). Only healthy individuals of similar size (20–50mg) were used in the bioassays.

2.2. Pesticides and Mixture Treatments

Three commercial pesticides were used:

Pesticide	Type	Active Ingredient	Formulation	Conc. Range Tested
Cyperkill	Insecticide	Cypermethrin (pyrethroid)	EC 10%	0.15, 0.30, 0.60 ppm
Garlon	Herbicide	Triclopyr	SL 48%	300, 600, 900 ppm
Benlate	Fungicide	Benomyl (carbamate)	WP 50%	300, 600, 900 ppm

2.3. Combined Mixtures

Binary combinations were prepared using a fixed-ratio design at ecologically relevant doses:

- Cyperkill + Garlon (0.15 + 600 ppm)
- Cyperkill + Benlate (0.15 + 600 ppm)
- Garlon + Benlate (300 + 600 ppm)

All treatments were freshly prepared using distilled water before application.

2.4. Chronic Exposure

Pesticide solutions were applied to dry leaf litter and thoroughly air-dried. Each treatment group consisted of 30 isopods (10 per replicate × 3 replicates), housed in plastic containers (12 × 12 × 5 cm) containing 50 g of treated soil and 2 g of treated leaves. Control groups received distilled water only. The test duration was 8 weeks, during which the following parameters were assessed weekly or bi-weekly:

2.5. Acute Toxicity Bioassay – LC_{50} Estimation

To estimate LC_{50} values for Cyperkill, a separate acute toxicity test was conducted using five concentration levels (1.25, 2.5, 5, 10, 20 ppm) on filter paper-lined Petri dishes. Each dish received five isopods and 1 ml of pesticide solution. Mortality was recorded after 24 and 48 hours. Probit regression was applied using SPSS to determine LC_{50} values and confidence intervals.

2.6. Statistical Analysis

All data sets were tested and confirmed to follow a normal distribution. Accordingly, Student's t-test and one-way ANOVA were employed to evaluate statistically significant differences among treatment groups for key endpoints, including body weight change, food consumption, juvenile production, and mortality. Additionally, Probit regression analysis, following the classical method of Finney (1952), was used to calculate LC_{50} values for Cyperkill at 24-hour and 48-hour exposure periods under acute toxicity testing conditions.

3. Results

3.1. Body Weight

Chronic exposure to Cyperkill, Garlon, Benlate, and their binary combinations significantly impacted the body weight of *Porcellio laevis*. While control animals showed a positive weight gain (0.229 ± 0.095 mg), most pesticide-exposed groups exhibited weight loss (See Supplementary Figure S1 for additional replicates and raw data), with the most pronounced decrease observed in the Cyperkill + Garlon mixture (–0.303 ± 0.050 mg) (Figure 1).

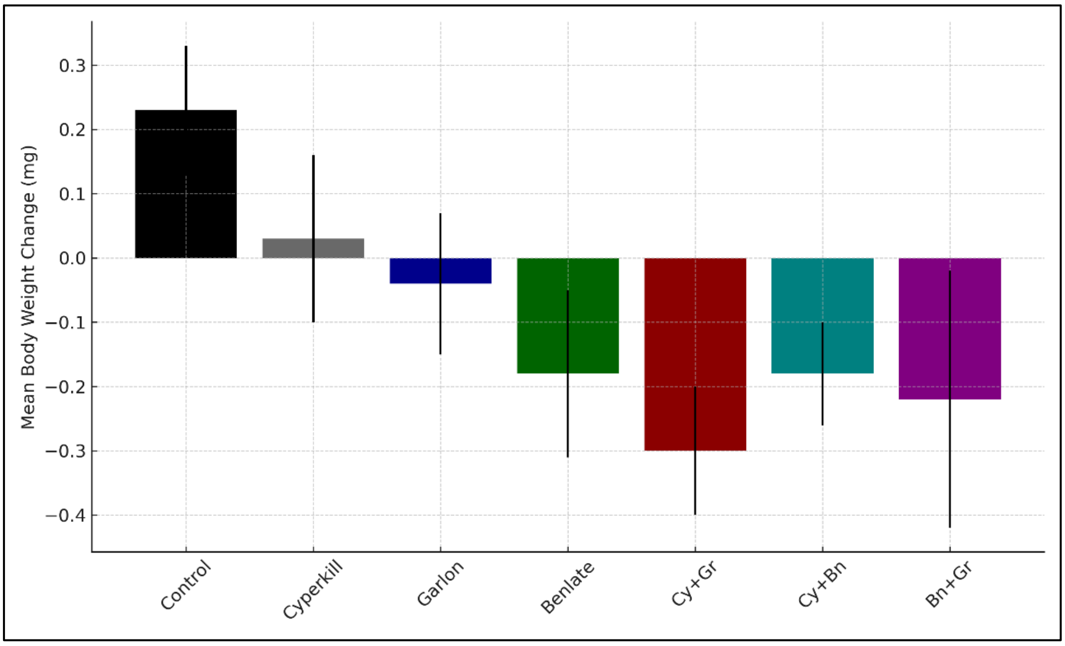

Figure 1 Average body weight change of *Porcellio laevis* after 28-day exposure to single and combined pesticide treatments

All pesticide-exposed groups showed reduced body mass compared to the control, with the most significant reductions occurring in the Cyperkill + Garlon and Benlate + Garlon treatments, suggesting possible synergistic toxicity.

Benlate alone induced a significant weight loss across all tested concentrations (mean = –0.183 ± 0.118 mg), followed by Garlon (–0.043 ± 0.197 mg). Cyperkill alone had a minimal effect on body weight (0.033 ± 0.130 mg), but in combination with other pesticides (especially herbicide Garlon), the effect became synergistically more toxic. Significant differences were observed between treatments (F = 7.237, P < 0.001). However, the pesticide concentration level alone did not account for significant variance (F = 1.70, P = 0.194), suggesting that the type of compound and its interactions play a more critical role than dosage alone.

3.2. Food Consumption

Food consumption by *Porcellio laevis* was affected by pesticide exposure (Detailed feeding trials are presented in Supplementary Figure S2), though the differences were not statistically significant across treatments (F = 2.057, P > 0.05). Control groups consumed the highest amount of food (3.91 ± 0.33 mg), followed by Cyperkill (3.08 ± 0.23 mg), and Benlate (2.86 ± 0.23 mg). The lowest food intake was observed in the Garlon + Benlate combination (2.42 ± 0.17 mg) (Figure 2).

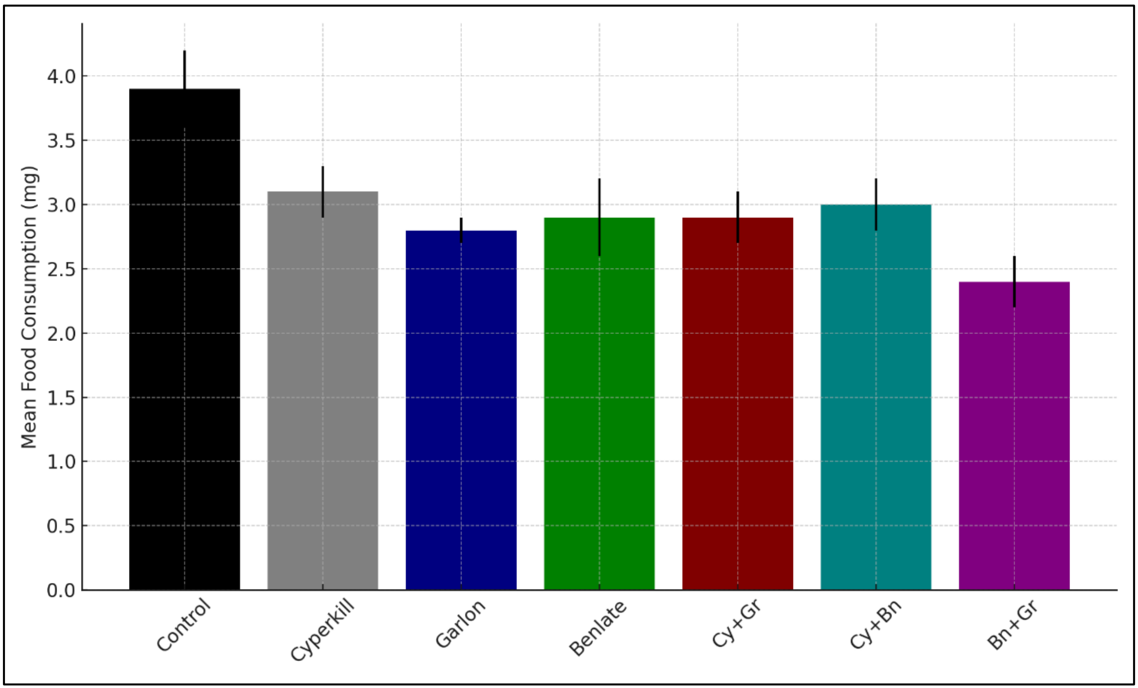

Figure 2 Feeding rates (mg consumed) of *Porcellio laevis* under different pesticide exposures

Food intake was highest in the untreated control, while notable declines were observed in all treatment groups, particularly under the Benlate + Garlon combination, indicating potential interference with normal feeding behavior.

Despite the absence of statistically significant differences, trends suggest reduced food consumption in pesticide-exposed groups, especially under binary mixtures. The Cyperkill + Benlate (2.99 ± 0.19 mg) and Cyperkill + Garlon (2.87 ± 0.24 mg) mixtures showed intermediate values, while individual treatments with Garlon and Benlate alone resulted in slightly lower consumption than Cyperkill. These findings suggest that feeding behavior may be subtly altered by pesticide exposure, likely due to its repellent or physiological stress effects.

3.3. Juvenile Production

Reproductive output in *Porcellio laevis* declined notably in pesticide-exposed groups (Supporting reproduction data can be found in Supplementary Figure S3). The control group showed the highest juvenile production (12 juveniles per group), followed by Cyperkill (9) and Benlate (8). The lowest reproductive success was observed in binary mixtures, particularly Benlate + Garlon (3 juveniles) and Cyperkill + Garlon (4 juveniles), as shown in Figure 3.

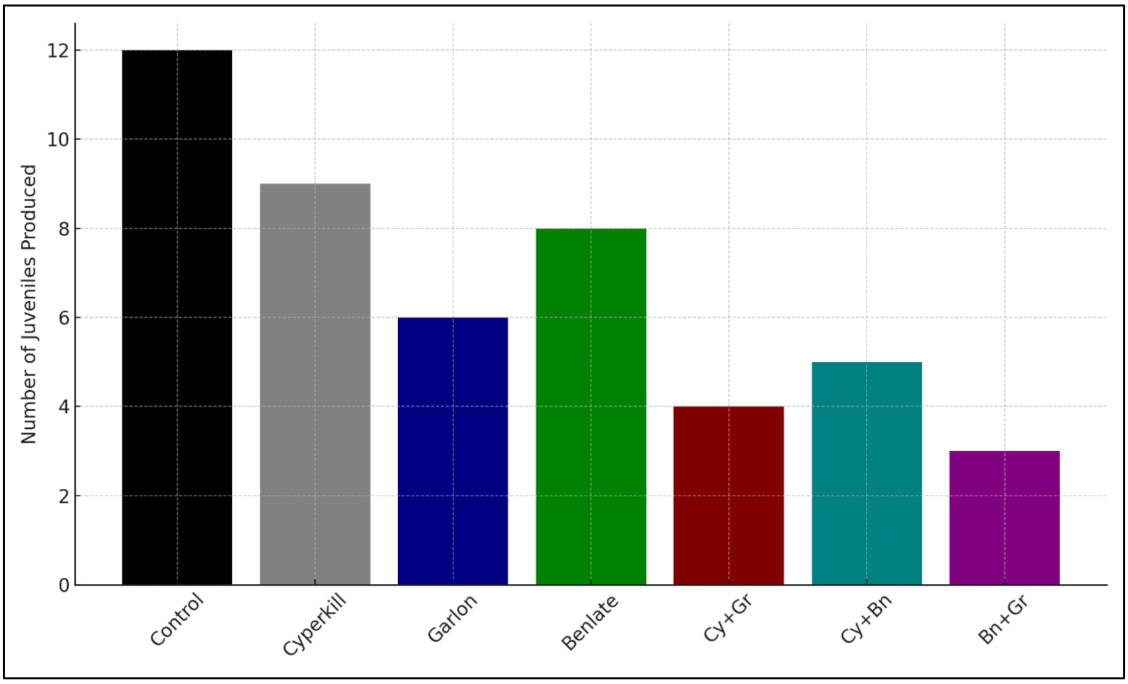

Figure 3 Juvenile production by *Porcellio laevis* under single and combined pesticide exposures

The number of offspring was most significant in the control group. Reproductive performance declined significantly in all treated groups, with the lowest output recorded in the Benlate + Garlon group, highlighting the adverse reproductive impacts of pesticide mixtures.

Although no statistical test was conducted due to limited replication in reproduction data, the trend clearly demonstrates reduced fertility or survival of offspring under chronic pesticide stress. This result supports the hypothesis that pesticide combinations can exert synergistic reproductive toxicity, further compromising population viability.

3.4. Mortality

Mortality among *Porcellio laevis* exposed to various pesticides over the 8-week period varied significantly across treatments (See Supplementary Figure S4 for mortality). The control group showed 0% mortality, confirming the environmental suitability of the test conditions. Among single pesticides, Cyperkill exhibited the highest mortality rate (33%), followed by Garlon (20%) and Benlate (12%).

Binary mixtures produced elevated mortality levels, with the highest mortality recorded in the Cyperkill + Garlon group (45%), followed by the Benlate + Garlon group (43%) and the Cyperkill + Benlate group (40%) (Figure 4). These findings indicate synergistic interactions between pesticide pairs, as mortality rates in combinations exceeded the expected additive effects of individual compounds. The observed pattern suggests that pesticide interactions in soil ecosystems may lead to underestimated risks if single-compound toxicity is considered.

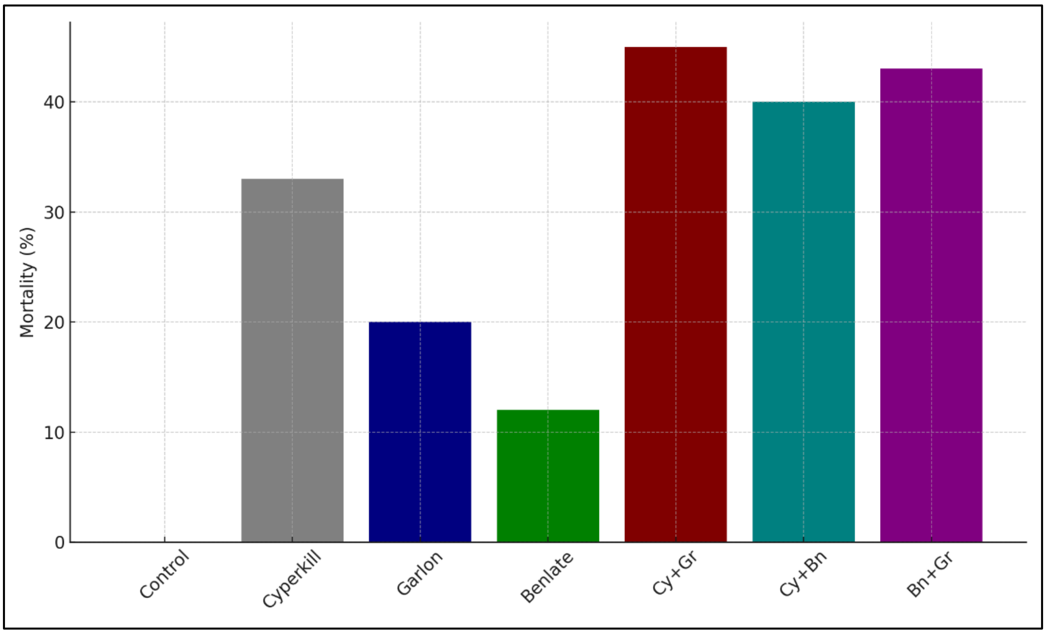

Figure 4 Observed mortality rates of *Porcellio laevis* after 28-day pesticide exposure

Pesticide-treated groups experienced elevated mortality compared to the control, with the highest death rates recorded in groups exposed to Cyperkill in combination with either Garlon or Benlate, reflecting potential synergistic effects.

3.5. Acute Toxicity (LC_{50}) of Cyperkill

Acute exposure trials revealed that Cyperkill has a highly toxic profile toward *Porcellio laevis*. The LC_{50} at 24 hours was calculated at 0.483 ppm, while the 48-hour LC_{50} was lower, at 0.289 ppm, indicating increased lethality over time.

The Probit analysis confirmed dose-dependent mortality, with well-fitted cumulative mortality responses over the log-transformed concentrations (Figure 5). The mortality curve shifted leftward over time, reflecting the time-dependent potency of Cyperkill (Probit regression charts and raw mortality data are included in Supplementary Figure S5).

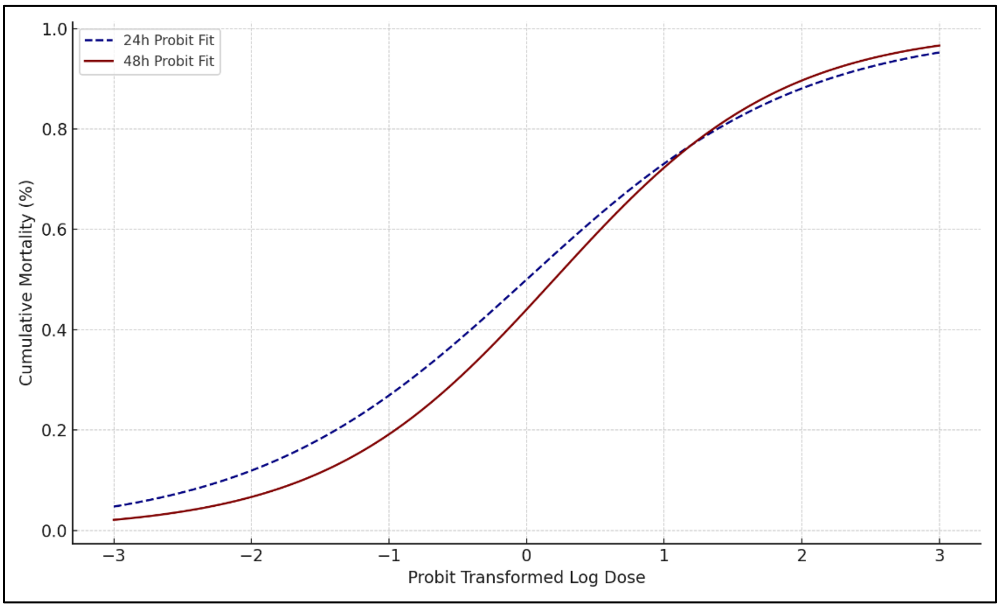

Figure 5 Probit analysis of Cyperkill toxicity showing LC_{50} estimates at 24 and 48 hours

Dose-response curves display cumulative mortality (%) as a function of probit-transformed log doses. Increased toxicity was observed with longer exposure time, with the 48-hour curve indicating a lower LC_{50} than the 24-hour curve.

These LC_{50} values indicate that Cyperkill exhibits extreme toxicity to *Porcellio laevis* under controlled laboratory conditions, a finding consistent with the known neurotoxic effects of pyrethroids. These effects include the disruption of sodium channel kinetics in neural membranes, causing prolonged excitation and eventual failure of neuromuscular systems.

4. Discussion

The present study demonstrates that low concentrations of three widely used agrochemicals, Cyperkill (insecticide), Benlate (fungicide), and Garlon (herbicide), have both chronic and acute toxic effects on the terrestrial isopod *Porcellio laevis*. These effects were magnified when pesticides were used in binary mixtures, often resulting in synergistic outcomes in mortality and sublethal endpoints such as body weight, food consumption, and reproduction.

4.1. Chronic Exposure and Sublethal Effects

Over 8 weeks of feeding, the isopods showed clear physiological and reproductive impairments when exposed to pesticides. Benlate and Garlon treatments caused a significant reduction in body weight, whereas Cyperkill had minimal effects when administered alone. However, all binary combinations (Cy+Gr, Cy+Bn, Bn+Gr) led to more pronounced body weight loss, with Cyperkill + Garlon being the most severe, indicating interactive toxicity beyond simple additive effects.

Food consumption was also reduced in all pesticide-treated groups compared to the control, although the differences were not statistically significant. This suggests potential behavioral avoidance [27] or feeding deterrence possibly induced by pesticide residues in the food matrix. The highest feeding occurred in the control group, while the lowest was recorded for the Gr+Bn combination. Reduced food intake likely contributed to the observed decline in body weight.

Reproductive success, as measured by juvenile count, also declined in all pesticide-treated groups, with the most substantial decrease observed in isopods exposed to Cyperkill + Benlate and Benlate + Garlon mixtures. This highlights the long-term reproductive risks [2] posed by chronic low-dose exposure to pesticide combinations.

These findings are supported by [5], who reported negative growth efficiencies and reduced assimilation in isopods exposed to contaminated substrates. The pattern of negative weight gain and impaired feeding efficiency under pesticide exposure is consistent with the idea that chronic toxicity manifests subtly but significantly over time in detritivores such as isopods.

4.2. Acute Toxicity and LC_{50} Dynamics

Acute toxicity trials with Cyperkill alone revealed a 24-hour LC_{50} of 0.483 ppm and a 48-hour LC_{50} of 0.289 ppm, confirming the high neurotoxicity of this pyrethroid to *Porcellio laevis*. These LC_{50} estimates, calculated using classical Probit methodology [26], demonstrate the reliability of dose–response modeling in ecotoxicology. The drop in LC_{50} over time suggests a cumulative toxic load, consistent with evidence that toxicological thresholds such as IC_{50} values can shift with exposure duration and recovery time [25], which is likely linked to Cyperkill's known mechanism of action, disruption of voltage-gated sodium channels in nerve membranes [21]. Compared to earlier findings [28], the LC_{50} values here further solidify Cyperkill's status as super-toxic to non-target soil fauna, especially when bioavailability in leaf litter and soil is optimized.

4.3. Pesticide Interactions and Synergism

Binary mixtures consistently exhibited greater toxicity than individual pesticides, particularly in mortality and weight loss. This supports the view that mixture toxicity cannot be inferred from single-compound data and aligns with the principles of Concentration Addition (CA) and Independent Action (IA) models in ecotoxicology.

While Garlon and Benlate individually showed moderate or low toxicity, their combination with Cyperkill resulted in outcomes indicating synergistic interaction, consistent with the findings of [5], who demonstrated antagonism at low concentrations and synergism at high concentrations in soil fauna exposed to pesticide mixtures.

4.4. Ecological Implications

The cumulative evidence suggests that even non-lethal pesticide concentrations can compromise key biological traits in soil detritivores such as isopods [16,29,30]. These organisms are not only critical for litter decomposition and nutrient cycling but also serve as early warning indicators of ecological imbalance.

While mortality may be a conspicuous endpoint, sublethal traits such as growth, feeding, and reproduction serve as more sensitive indicators of ecological stress in chronic exposure scenarios. Furthermore, binary interactions exacerbate risk, underscoring the need for ecological risk assessments to account for chemical mixtures, not just individual agents.

5. Conclusion

This study highlights the amplified ecotoxicological effects of binary pesticide mixtures on a key soil decomposer. It highlights the need to assess not only acute toxicity but also sublethal and reproductive impacts in environmental risk assessments. *Porcellio laevis* proves to be a suitable bioindicator for such evaluations.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed

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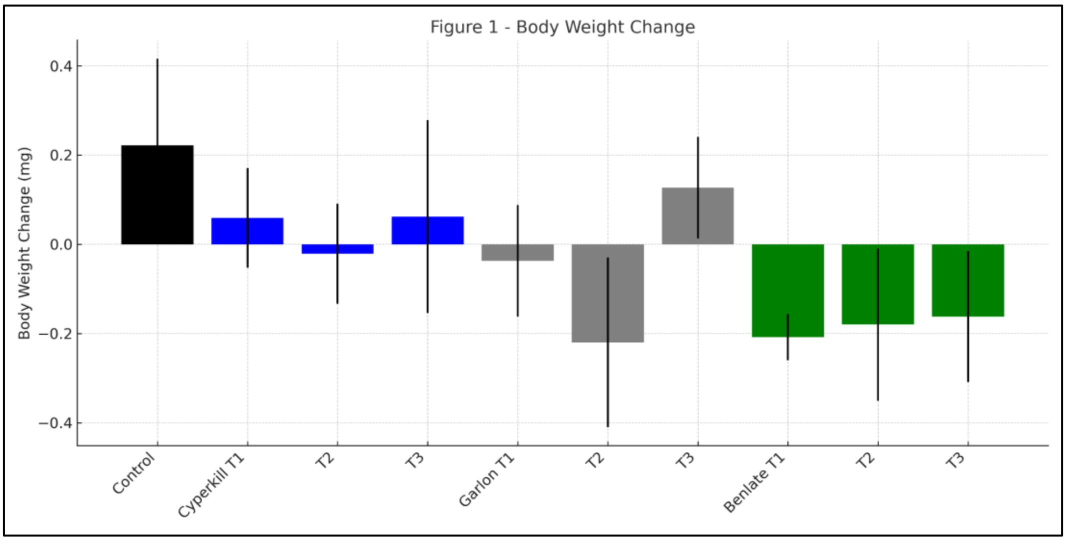
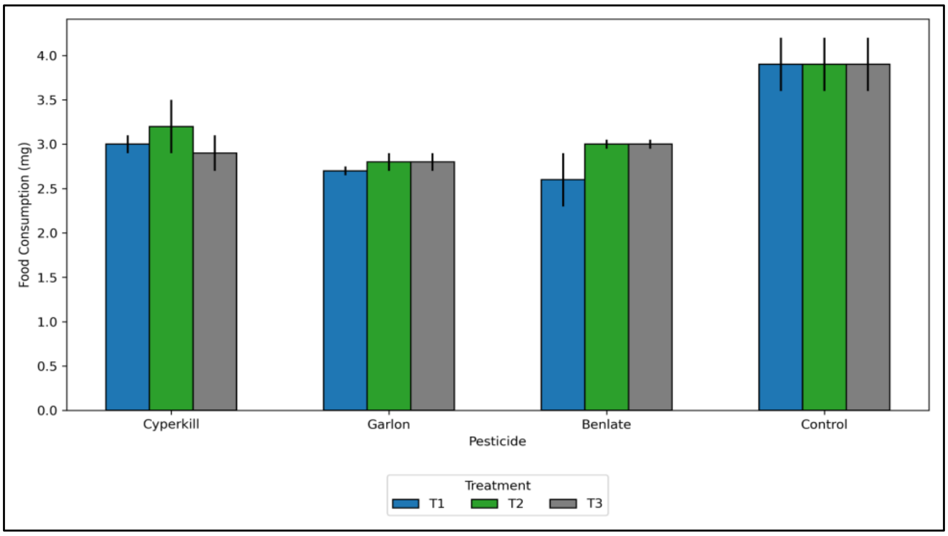
Supplementary Material – Dose-Dependent Responses of *Porcellio laevis* to Individual Pesticides

This figure shows how each pesticide individually influenced the growth patterns of isopods at increasing concentrations. Garlon and Benlate caused notable weight loss compared to controls.



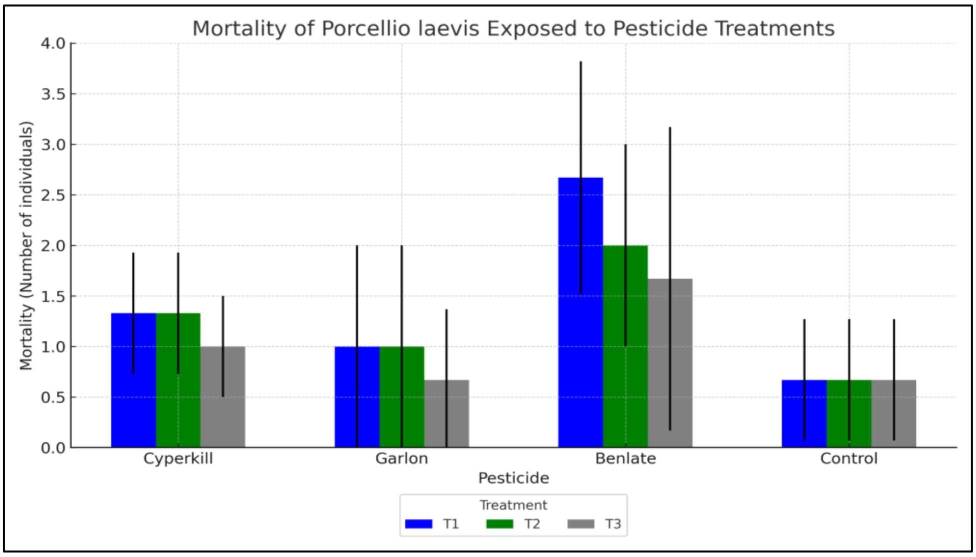
Supplementary Figure S1. Body weight changes in *Porcellio laevis* after 8-week exposure to different concentrations (T1, T2, T3) of Cyperkill, Garlon, and Benlate. Data presented as mean ± SD.

This figure illustrates the trend in feeding activity across pesticide types and concentrations. A decline in consumption is especially evident under Garlon exposure.



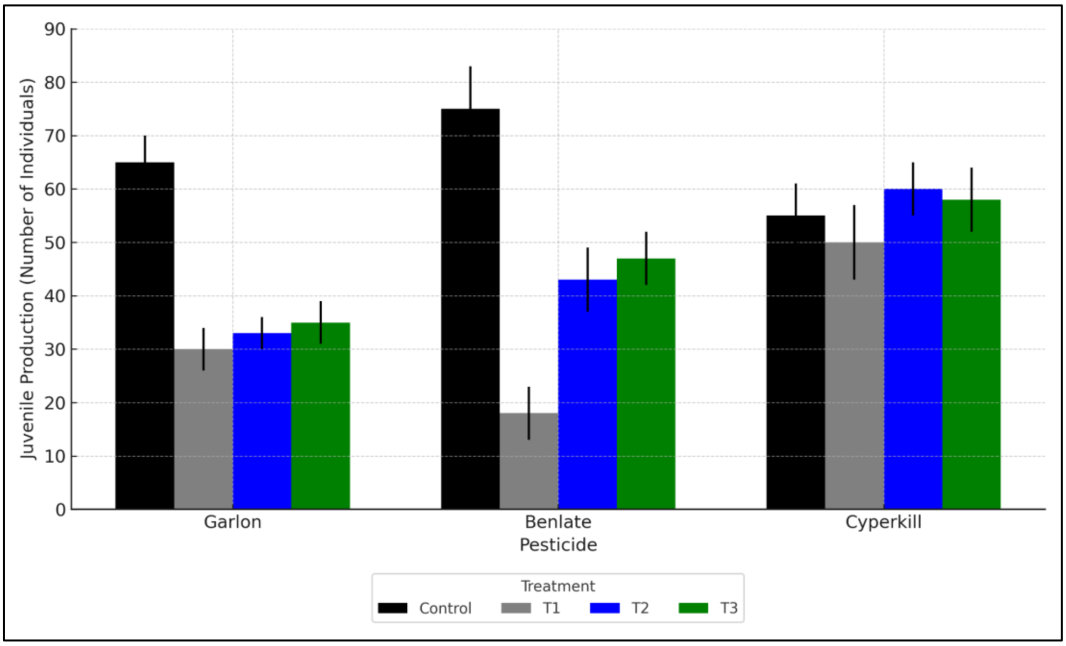
Supplementary Figure S2 Food consumption (mg) by *Porcellio laevis* after 8-week exposure to increasing concentrations (T1, T2, T3) of Cyperkill, Garlon, and Benlate. Error bars represent SD.

This mortality plot confirms dose-response trends, with higher mortality under Benlate and Garlon treatments, especially at T2 and T3.



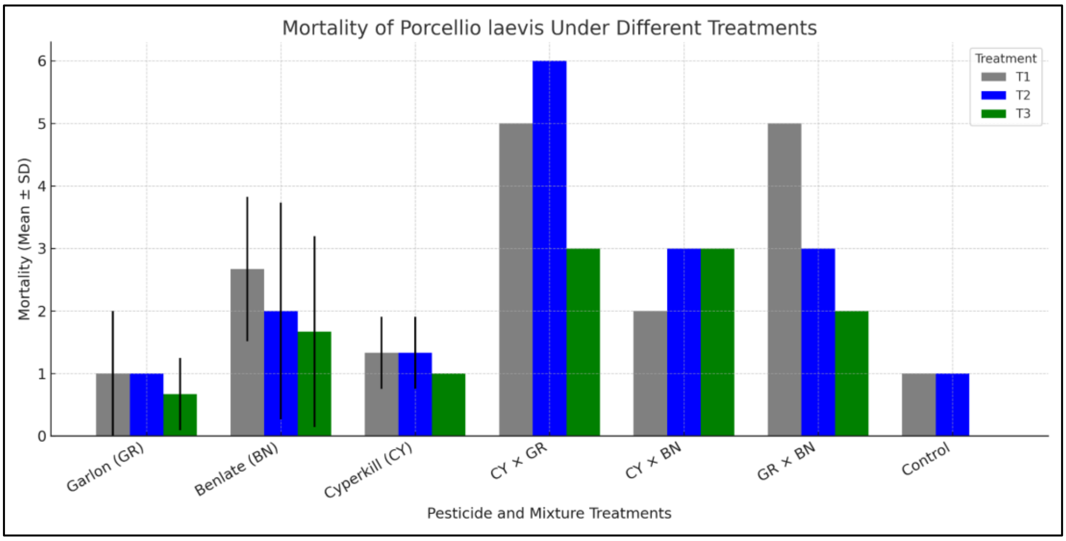
Supplementary Figure S3. Mortality rates (%) of *Porcellio laevis* under different concentrations (T1, T2, T3) of Cyperkill, Garlon, and Benlate over 8 weeks. Error bars indicate SD.

This figure emphasizes how reproductive output sharply declined under Benlate and Garlon exposure, with Cyperkill showing moderate effects.



Supplementary Figure S4. Juvenile production (number of offspring) by *Porcellio laevis* exposed to individual pesticides at three concentration levels. Error bars represent SD.

This figure presents the synergistic impact of combined pesticide exposures, highlighting the elevated toxicity of Cyperkill + Garlon.



Supplementary Figure S5. Mortality response of *Porcellio laevis* to binary pesticide combinations at three treatment concentrations (T1–T3). Data are mean ± SD.