



Review Article

Importance of red beans in artificial diets for *Troides helena* (Linnaeus) larvae: A review

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ARTICLE INFORMATION



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Citation: Aminah, S. N. (2025). Importance of red beans in artificial diets for *Troides helena* (Linnaeus) larvae: A review. *Wallacea Plant Protection Journal*, 1(2), 59–64.
<https://doi.org/10.64128/wppj.v1i2.47262>

Editor handling: Ito Fernando

Received : 20 September 2025
Revised : 26 December 2025
Accepted : 30 December 2025
Published : 06 January 2026

ABSTRACT

The common problem in insect artificial diet for Lepidopteran, especially *Troides helena* (Linnaeus) (Lepidoptera: Papilionidae) larvae is preparing wheat germ to cover the nutritional needs of the insect target. All of the material formulated in the artificial diet has different textures, water content, and nutritional composition compared to the natural diet from the butterflies host plant, *Aristolochia* leaves. The ability to choose and control the nutritional component of the artificial diet gives the best results for the mass-rearing of insect targets. Larvae of *T. helena* have been successfully mass-reared in artificial diet-based red beans as a substitute for wheat germ. Artificial diet incorporating red beans or kidney beans have been shown to increase longevity and the successful emergence of the adults from the pupal stage. An artificial diet was given to *T. helena* larvae from the 2nd instar until they formed a pupal. Previous research showed that *T. helena* adults, while hatching normally a males, often remained crippling butterflies. In the future, the artificial diet of butterflies is the solution increasing population of insects in Bantimurung-Bulusaraung National Park, South Sulawesi.

Keywords: Bantimurung-Bulusaraung; Butterfly; Conservation; Insect nutrition; Mass-rearing; Wheat germ substitute

1. Introduction

Research on insect biology must be done very carefully because of the differences in insect needs including behavior in terms of habitat selection, host plants eaten, copulation, ability to produce eggs, and others. Regarding the type of food consumed, each insect pest has its host plant, respectively. Commonly, insect are beneficial for increasing human health, as stated by (Olowoyeye, 2025; Weidner et al., 2020).

Related to beneficial insect management, the use of artificial diets in conservation is particularly important for endangered or ecologically significant insect species. For example, butterflies such as *Troides helena*, listed under CITES Appendix II and protected by Indonesian law, require specialized diets for successful captive breeding. A study conducted in Bantimurung-Bulusaraung National Park, South Sulawesi, demonstrated that red bean-based diets resulted in a 57.05% survival rate for *T. helena* larvae, outperforming mung bean sprouts and approaching the effectiveness of natural host plants (Aminah et al., 2014). Red beans are rich in proteins, carbohydrates, and essential micronutrients, making them suitable for insect diets. Their affordability and availability in tropical regions like Indonesia make them an attractive alternative to imported

ingredients. A recent study evaluated the nutritional physiology and digestive function of *Helicoverpa armigera* larvae fed on diets containing various legumes, including red kidney beans. The results showed high efficiency of conversion of digested food (ECD) and relative growth rate (RGR), indicating that red beans can support larval development effectively (Altermatt, 2010; Wang et al., 2021).

Moreover, red beans have shown potential in improving the emergence rates and reproductive success of insects. In the *T. helena* study, male emergence from red bean diets reached 71.20%, and female emergence was 48.08%, suggesting that red beans can support both survival and reproduction in conservation contexts (Aminah et al., 2014). Artificial diets offer several advantages over natural feeding such as: nutritional composition can be standardized, reducing variability in insect development, the ingredients like red beans are accessible year-round, unlike seasonal host plants, local grains reduce dependency on expensive imports, the artificial diets simplify rearing protocols and minimize handling risks and suitable for large-scale conservation and research programs (Akinneye & Ashamo, 2009; Mukhametov et al., 2023; Ryne et al., 2004).

This manuscript is based literature review. Various databases are used here such as: Scopus, Web of Science, Google Scholar, ProQuest, PubMed and the Directory of Open Access Journals (DOAJ). Scientific articles were searched using the following keywords: 1) “insect artificial diet” OR “butterfly” OR “wheat germ” OR “red bean” OR “conservation” OR “mass-rearing”. The selected articles then went through the subject screening stage to remove the articles from the unsuitable subject (Novita et al., 2023) with modification.

2. Challenge on Artificial Diet Preparation

Natural foods from the host typically contain a higher density of nutrients per calorie, meaning they provide more vitamins, minerals, and other beneficial compounds with fewer calories. The study of Rojas & Morales-Ramos (2010) used red bean leaves as the main food source of two-spotted spider mites (*Tetranychus urticae*). The mass-rearing of insects has been and continues to be a crucial part of entomological research. Cohen (2001) reported that more than 60% of research reports written about studies involving insects use artificially reared insects rather than field-collected populations. Approximately 45% of those insects were reared on artificial diets rather than on their natural diet. When insects are reared on artificial diets, it implies that a synthetic diet is provided instead of what it would normally consume, without affecting their growth and development.

Pinto et al. (2019) and Vanderzant (1969) state that the laboratory colonies of insects reared on artificial diets reduce the labor, time, space, and in many cases, the high costs associated with rearing insects on their natural host plants. It is not always feasible to keep insects on their natural host plants because of seasonal availability, high costs, and varying quality of host plants over time.

In many cases, however, the host-plant factor can be incorporated into the artificial diet. Commonly, artificial diets have been formulated based need for insect nutrition in mass-rearing and their economic importance (rare species under government regulation). Sørensen et al. (2012) state that several advantages of insect artificial diet, such that suitable for insect target nutritional needs, available at any time without being influenced by the season, being able to be produced in large quantities, lasts long in storage, being able to be combined with other formulas, and is free of pests and diseases. The cost used to provide artificial diets is said to be expensive because most of them use imported ingredients when compared to natural hosts available in nature.

In the effectiveness of mass-rearing activities, insects that have high economic value should be used. Mass-rearing beneficial insects or pest insects (as the prey or host) in laboratory conditions is a main component of several integrated pest management strategies. However, the activities have dramatic effects on insect performance via laboratory adaptation, inbreeding depression, and direct rearing effects on artificial diets. Moore et al. (2014) reported that the main problem in mass rearing insects is preparing the environment such as their natural habitat and food containing nutrition for development. This kind of natural material has different insects to other species.

Access to the host plant as the food source cannot be discontinued for successful insect mass-rearing.

3. Importance of Red Beans as a Material Substitute

Commonly on previous research, insect artificial diet for the butterfly immature stage uses wheat germ as an important source of protein (Lisboa et al., 2025; Mahmoud et al., 2015; USDA, 2019). Unfortunately, wheat germ is very expensive and difficult to buy. It is the main reason the insect mass-rearing technique develops well using many substitute materials including red beans in the artificial diet. The composition of red beans and wheat germ as the material in the artificial diet for butterfly larvae is shown in Table 1.

Table 1. Composition of red beans and wheat germ in the artificial diets for butterfly larvae

Composition	Red Beans *	Wheat Germ **
Carbohydrate (g)	70.48 ± 0.14	52.73
Protein (g)	23.00 ± 0.03	31.69
Lipid	1.38 ± 0.26	10.29
Ash	5.13 ± 0.09	3.53
Dietary fiber	20.93 ± 0.23	13.20
Energy value (kcal)	386.39 ± 1.60	360.00

* Sahasakul et al. (2022)

** Mahmoud et al. (2015)

As a substitute for wheat germ, red beans are rich in carbohydrates and protein useful for increasing the fitness of the insect target. Téllez-Mazzocco et al. (2025) have been characterized five wild *Phaseolus* species and found significant variation in protein, polysaccharide, and lipid content, highlighting their potential for nutritional optimization in insect diets. Defilippo et al. (2025) reported that they formulated an artificial diet using soy flour, casein, and yeast, successfully rearing five dipteran species. Their results showed comparable development rates to natural diets, emphasizing the importance of standardized, shelf-stable ingredients better the use of bio material such as beef liver and pig muscle. Table 2 shows the material, amounts and diverse insect target in artificial diet based on the red beans.

Table 2. Material, amount, and lepidopteran species used in formulating artificial diets

Material	Amount (g)	Lepidopteran species
Kidney bean flour *	51.3	<i>Spodoptera litura</i>
Red bean flour **	6.0	<i>Troides helena</i>
Red bean flour ***	6.0	<i>Hypolimnas bolina</i>
Red kidney flour ****	7.5	<i>Conopomorpha cramerella</i>

* Gupta et al. (2005)

** Aminah et al. (2014)

*** Kurnia et al. (2021)

**** Niogret et al. (2023)

The increasing demand for sustainable insect rearing practices has led to the exploration of alternative

ingredients for artificial diets. Red beans (*Phaseolus* spp.), known for their rich protein and nutrient content, present a promising substitute for conventional diet components and a possible substitute for wheat germ (Meng & Ma, 2002). Based on Table 2, not only is beneficial insect rearing used with an artificial diet. For example, this pest insects in Table 2 often occurs in mixed populations in cereal crops and can cause yield losses ranging from 10% to total crop loss. The reason sometimes insect pests reared in the artificial diet because they are prey of arthropods natural enemies, especially predators. The abundance and highly similar performances will succeed mass-rearing predators in the laboratory research on these pests necessitates rearing on artificial diets to ensure reliable supplies of high-quality insects for increasing the fitness of natural enemies. High-quality insects are also needed for the rearing of biocontrol agents. Based on research by Ali et al. (2024), Blanco et al. (2009), Hervet et al. (2016), Pereira et al. (2010), and Truzi et al. (2021) stated that soybean flour and wheat germ are the two most important protein components of wheat germ-based insect artificial diets. The effect of modifying the proportion of these two ingredients in a Noctuidae-specific diet was investigated utilizing the tobacco budworm *Heliothis virescens*, to develop a suboptimal diet that, without drastically affecting insects growth and reproductive rates, could manifest negative effects in insect target. Related presence legum used in the insect artificial diet, research of Ahmed et al. (2023), Chen et al. (2022), and Truzi et al. (2021) have tested bean and chickpea flour-based diets for *Spodoptera frugiperda* and found that chickpea flour supported better growth and fecundity, suggesting legumes suitability for insect rearing. Strydom et al. (2024) evaluated diets for six Lepidopteran pests and found that sugarcane and maize leaf-based diets were optimal for specific species. Their study underscores the need for species-specific diet formulations. The result research of Téllez-Mazzocco et al. (2025) reported that the material contains antinutritional factors (lectins and tannins) can be changed by processing techniques such as soaking, boiling, or fermentation for safety application. Standardization of bean flour quality and consistency is also essential for reproducible results in insect artificial diet.

4. Mass-Rearing *Troides helena* Larvae in an Artificial Diet

Insect artificial diets are essential tools for arthropods research. These diets have been formulated to maximize insect growth and reproduction. Therefore, Blanco et al. (2009) and Niogret et al. (2023) state that these diets may mask potential developmental negative effects when they are used for reproductive studies that would be apparent only when the insects are exposed to sub-optimal diets. The common problem with insect artificial diet especially for butterfly larvae is the nutritional composition of the material used. The artificial diet have very different textures, water content, and nutritional composition compared to the natural diet from *Aristolochia* leaves. Aminah et al. (2014) reported that *T. helena* larvae mass rearing in *Aristolochia tagala* success 100% hatched a healthy butterfly, then artificial diet based red bean produced abnormal butterfly with crippling wings. Becher

& Guerin (2009) and Cahenzli & Erhardt (2012) reported that in the natural habitat, *Aristolochia* has bitter plant parts and produces aristolochic acid as a plant defense. This condition gives benefits to avoid herbivorous insects. The ability to control the nutritional component of the artificial diet gives the best results for the mass-rearing of insect targets. *T. helena* larvae have been successfully mass-reared in an artificial diet based on red beans as a substitute for wheat germ. Artificial diet-based red beans able to increase longevity and adults hatched from the pupa. Impact of artificial diet on stages and duration ranges of *T. helena* larvae from the 2nd instar until they formed a pupal showed in Table 3.

Table 3. Immature duration of *Troides helena* on red beans

Stages	Duration range (days) *
Larval	
2 nd instar	6–10
3 rd instar	7–9
4 th instar	7–8
Prepupal	2
Pupal	2–3

* Aminah et al. (2014)

Red beans contain essential amino acids such as lysine, leucine, and methionine, which are critical for insect growth and reproduction. Their fiber content aids digestion, while micronutrients support metabolic functions (Meng & Ma, 2002; Molleman et al., 2009; Strydom et al., 2024). The inclusion of red beans can reduce reliance on animal-derived ingredients and improve sustainability. Based on research results of *T. helena* larvae in Table 3 showed that the butterfly hatches normally male and remains a more crippling butterfly (Aminah et al., 2014). This condition, a side effect of the artificial diet, is similar to decreased reproduction ability observed in *Agrotis ipsilon* (Force et al., 2023) and highlights that the insect has specialized host plants that are suitable for development (Ramos et al., 2019). The solution of the mass rearing problem of *T. helena* larvae is adding other natural materials and mixing with red beans, increasing fitness of the butterfly. It needed more effort to deeply research related insect nutrition in larvae stage. The results from Aminah (2023), (2024), Kurnia et al. (2021), and Niogret et al. (2023) presented that red beans are a viable substitute for conventional ingredients in insect artificial diets from the order Lepidoptera. Their nutritional profile supports insect development, and recent studies validate their efficacy. Adoption of red bean-based diets can contribute to sustainable insect rearing practices. Further research should focus on optimizing red bean-based diet formulations for different insect in endangered status, assessing long-term effects on insect health and reproduction, and evaluating cost-effectiveness. Integration with other plant-based ingredients may enhance diet performance.

5. Conclusion

The conclusion of the study of red bean as potential component in the artificial diet is: 1) red beans are the nutritionally viable plant-based protein substitute (addressing the herbivory/cost issues) and 2) substitution requires further theoretical investigation, particularly regarding the need for species specific optimization to fully eliminate developmental abnormalities like crippling, also include future directions for insect mass rearing.

Author Contributions: Sri Nur Aminah: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: I am very grateful to the Dean of the Faculty of Agriculture, Hasanuddin University for permission of our independent research study.

Conflicts of Interest: The authors declare no conflicts of interest.

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