Acta Entomologica Serbica, 2026, 31(2): xx-xx

UDC:
DOI: 10.5281/zenodo.23543878

Article

BIOCONVERSION OF BROCCOLI WASTE BY *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The increasing generation of organic waste and growing pressure on natural resources have highlighted the need for sustainable approaches to resource utilization. In this context, insect-mediated bioconversion represents a promising strategy for transforming organic waste into nutrient-rich biomass. The present study investigated the potential of the cotton leafworm, *Spodoptera littoralis*, as a biological agent for biomass production, using broccoli florets from municipal organic waste as a rearing substrate. Freeze-dried *S. littoralis* larval biomass (generation F3) was chemically characterized by determining its proximate composition, mineral profile, and amino acid composition. In addition, biological parameters were evaluated across two generations to assess the consistency of larval development and reproductive performance under the tested rearing conditions. The biomass contained 48.04% protein and 17.33% lipids, while moisture, ash, and cellulose accounted for 8.09%, 7.52%, and 6.50%, respectively. Among the analyzed minerals, potassium was the most abundant element (49,062.04 mg/kg), followed by magnesium (3,673.14 mg/kg), sodium (2,126.11 mg/kg), and calcium (1,701.12 mg/kg). The amino acid analysis showed a total detected amino acid content of 35.84%, with glutamic acid (6.70 ± 0.25%) being the most abundant, followed by cysteine (4.12 ± 0.03%) and aspartic acid (3.33 ± 0.15%). Among the detected essential amino acids, leucine had the highest concentration (2.26 ± 0.15%), while valine was not detected. Biological parameters were comparable between the first and second generations, with no statistically significant differences detected for larval development duration, survival, larval mass, adult lifespan, or reproductive parameters. Overall, the results show that freeze-dried *S. littoralis* powder from insects reared on a waste-derived substrate contains substantial amounts of protein, lipids, minerals, and nutritionally relevant amino acids. These findings indicate the potential of using a well-known agricultural pest in controlled bioconversion systems and support further investigation of *S. littoralis* as a component of circular bioeconomy strategies.

KEYWORDS: insect biomass, circular bioeconomy, organic waste, nutritional composition, mineral composition, protein, sustainable resource utilization

Introduction

The rapid growth of the global population, coupled with increasing pressure on natural resources and the continuous generation of large quantities of organic waste, has intensified the need for more sustainable approaches to resource management (Makkar *et al*., 2014). In this context, the circular bioeconomy offers a way to use biological resources more efficiently. It focuses on reducing waste and using by-products and organic waste to produce useful materials and products. In this way, it can help reduce environmental impacts, improve resource use, and support more sustainable production (Hamam *et al*., 2024).
Among the proposed alternatives, edible insects have emerged as a promising source of nutrients due to their ability to efficiently convert organic substrates into biomass rich in proteins, lipids, minerals, and other valuable nutrients (Rumpold & Schlüter, 2013; Makkar *et al*., 2014). Their relatively rapid growth, short life cycles, efficient feed conversion, and ability to utilize a wide range of organic substrates further contribute to their potential as an efficient component of sustainable food and feed systems (Vantomme *et al*., 2014; Pinotti & Ottoboni, 2021). The nutritional composition of insect biomass can vary considerably depending on species, developmental stage, environmental conditions, and particularly the composition of the rearing substrate, suggesting that the selection and optimization of feeding substrates may provide an opportunity to influence the nutritional quality of the resulting biomass (Nowak *et al*., 2016; Pinotti & Ottoboni, 2021). A remarkable diversity of insect species, including moths, cicadas, beetles, mealworms, flies, grasshoppers, and ants, has been traditionally consumed across different regions and cultures worldwide (Ramos-Elorduy, 2009). The continuous increase in global resource consumption raises concerns regarding the long-term sustainability of current production models. In 2008, global human demand on natural resources was already estimated to exceed the Earth's regenerative capacity by approximately 30%, highlighting the need for more efficient and sustainable use of available resources (Hails *et al*., 2008).
Depending on the species and developmental stage, insects can be utilized throughout their life cycle, including as eggs, larvae, nymphs, pupae, or adults (Borror *et al*., 1976). Many insects can be farmed at relatively low economic and environmental costs; insect farming uses up to 50-90% less land per kg of protein and 40-80% less feed per kg edible weight, and produces 1,000-2,700 g less greenhouse gas emissions per kg mass gain than conventional livestock (Vantomme *et al*., 2014).

Insect biomass is generally characterized by a high content of proteins, lipids, essential amino acids, vitamins, and minerals, making insects a promising alternative source of nutrients compared with conventional animal-derived products (Rumpold & Schlüter, 2013; Makkar *et al*., 2014). Furthermore, certain insect species have been reported to contain considerable amounts of specific micronutrients that are less abundant in some traditional protein sources. For instance, termites are recognized as a valuable source of riboflavin, while silk moth larvae and palm weevils have been reported to contain high concentrations of thiamine, exceeding the levels found in chicken meat (Finke, 2002). Similarly, mealworms (*Tenebrio molitor*) exhibit a favorable nutritional profile, including high-quality protein containing all essential amino acids, as well as significant amounts of minerals and vitamins, with some nutritional parameters comparable to or exceeding those of conventional livestock products (Bukkens, 1997; Rumpold & Schlüter, 2013).

In addition to their nutritional benefits, insects represent an environmentally efficient alternative to conventional livestock production systems. Due to their biological characteristics, including rapid growth, efficient feed conversion, and the ability to utilize a variety of organic substrates, insect farming can significantly reduce the demand for land and feed resources while generating lower greenhouse gas emissions. Compared with traditional livestock, insect production may require up to 50-90% less land per kilogram of protein and 40-80% less feed per kilogram of edible biomass, while greenhouse gas emissions can be substantially reduced (Vantomme *et al*., 2014). Previous studies have also demonstrated that greenhouse gas emissions associated with insect production are considerably lower than those reported for livestock species such as cattle,

highlighting the potential role of insects within sustainable production systems and circular bioeconomy strategies (Oonincx *et al*., 2010).

The cotton leafworm, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae), is a highly polyphagous pest capable of feeding on approximately 90 economically important plant species from 40 different families. During the larval stage, it attacks a wide variety of field crops, vegetables, and ornamental plants, frequently resulting in considerable agricultural and economic losses. The species is widely distributed, with particular importance as an agricultural pest in Egypt and other regions worldwide (Ismail *et al*., 2012). Despite its conventional classification as an agricultural pest, the remarkable capacity of *S. littoralis* caterpillars to convert plant material into insect biomass suggests that this species may have potential applications beyond pest management, particularly within controlled bioconversion systems (El-Refaie *et al*., 2024).

Plant-derived residues often contain substantial amounts of carbohydrates, fibers, and other organic compounds that can serve as suitable nutritional resources for organisms involved in biomass transformation processes (Langeveld *et al*., 2018). Therefore, the selection of appropriate waste-derived substrates represents a crucial factor in optimizing insect growth and improving the quality of the resulting biomass. In the present study, freeze-dried biomass obtained from *S. littoralis* caterpillars reared on a substrate composed of broccoli florets from municipal organic waste was subjected to chemical characterization. The primary objective was to evaluate the basic nutritional composition of the produced biomass by determining the levels of major macronutrients, including proteins, lipids, and minerals. Such data may provide valuable information regarding the potential of insect-derived biomass as a renewable resource that could be further incorporated into circular bioeconomy approaches.

Materials and Methods

Insect origin and rearing conditions

The experimental population of the cotton leafworm, *Spodoptera littoralis* (Boisduval, 1833), originated from a closed laboratory colony established from insects purchased from Andermatt UK, part of the Switzerland-based Andermatt Group. The third and fourth laboratory generations of this colony, designated F3 and F4, were used in the experiment. The insects were maintained under controlled laboratory conditions at 22.5 to 23.5°C and 50-80% relative humidity, under a 16:8 h light:dark photoperiod. Larvae were reared in ventilated plastic boxes lined with sheets of unbleached kraft paper. The paper absorbed excess moisture and facilitated the removal of frass and uneaten plant material. It was replaced during box cleaning or whenever it became damp or heavily soiled.

Broccoli waste substrate

Broccoli florets were obtained through a formal memorandum of cooperation with AS VELRPO LLC, a local supply chain. The material represented broccoli that had lost its commercial value because the florets had become yellow. However, the florets showed no visible signs of spoilage or decomposition and were therefore considered suitable for use as a larval rearing substrate. Before being given to the larvae, the broccoli was visually inspected, and any unsuitable plant material or foreign matter was removed. The larvae were fed broccoli florets *ad libitum* throughout larval development. Fresh broccoli was added as required to ensure that food was continuously available. Uneaten broccoli, frass, and soiled kraft paper were regularly removed to maintain appropriate hygienic conditions within the rearing boxes.

Experimental design and monitoring

The biological performance of *S. littoralis* reared on broccoli waste was evaluated over two consecutive laboratory generations, F3 and F4. At the beginning of each generation, three independent replicate cohorts were established, each containing 100 second-instar larvae. Each generation therefore comprised 300 larvae, while the replicate cohort was considered the experimental unit. The cohorts were inspected daily, while detailed measurements were performed every second day. At each monitoring point, the number of surviving larvae and their total fresh mass were recorded. Average individual larval mass was calculated by dividing the total mass of living larvae by the corresponding number of larvae. Peak average larval mass was defined as the highest average individual mass recorded for each replicate cohort during larval development. Larval development duration was calculated as the number of days from the establishment of the second-instar cohorts until pupation. Larval survival was calculated as the percentage of the initial 100 larvae that successfully reached the pupal stage. When the larvae reached the prepupal stage, they were transferred to ventilated pupation boxes containing a layer of clean wood shavings. The wood shavings provided a loose substrate into which the larvae could burrow and pupate. The resulting pupae were maintained under the same laboratory conditions until adult emergence. Adult emergence was monitored daily. The numbers of emerging females and males were recorded separately for each replicate. Adults originating from the same replicate were maintained together and allowed to mate and oviposit, with 10% sucrose solution poured over cotton wool for their feeding. Egg masses were recorded throughout the reproductive period and classified according to their estimated size as small (<50 eggs), medium (50-200 eggs), or large (≥200 eggs). Total egg production was estimated separately for each replicate. Lifetime fecundity was calculated by dividing the total estimated number of eggs by the number of females. The offspring obtained from the F3 adults were maintained on the same broccoli substrate. After reaching the second larval instar, they were used to establish the three replicate cohorts of the F4 generation following the same experimental design and rearing procedure.

Preparation of insect biomass

A representative sample of F3 *S. littoralis* larval biomass was used for chemical characterization. The collected larvae were frozen and lyophilized until a stable dry mass was obtained. The lyophilized material was milled into a fine, homogeneous powder, hermetically sealed under vacuum, and stored at -24°C in airtight containers until analysis. Chemical analyses were performed in duplicate at the Institute of Food Technology, University of Novi Sad, Serbia, following the procedures described by Trajković *et al*. (2025).

Proximate composition

The proximate composition of the lyophilized larval biomass was characterized by determining moisture, crude protein, crude fat, ash, and crude cellulose contents. Crude protein was determined from 1 g of sample using the Kjeldahl method according to AOAC 978.04. A nitrogen-to-protein conversion factor of 6.25 was applied. Moisture was determined according to AOAC 934.01. Crude fat was determined from 5 g of sample by Soxhlet extraction according to AOAC 920.39, while ash was determined from 3 g of sample according to AOAC 942.05. Crude cellulose was determined gravimetrically according to SRPS EN ISO 6865:2008. Results were expressed as g/100 g of lyophilized biomass.

Amino acid composition

Amino acid composition was determined by ion-exchange chromatography using a Biochrom 30+ automatic amino acid analyzer (Biochrom, Cambridge, UK). Before analysis, 50 mg of each sample was hydrolyzed in 6 M HCl containing 0.1% phenol (Merck, Darmstadt, Germany) at 110°C for 24 h. Amino acids were separated

by strong cation-exchange chromatography and detected following the ninhydrin color reaction. Photometric detection was performed at 570 nm and at 440 nm for proline. Individual amino acid concentrations were expressed as g/100 g of lyophilized biomass. Tryptophan was not determined because it is destroyed during acid hydrolysis in 6 M HCl. Amino acids present below the detection limit of the method were reported as not detected (ND).

Mineral composition

The concentrations of calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) were determined by atomic absorption spectroscopy according to SRPS EN ISO 6869:2008. Samples were weighed and carbonized on a hot plate, followed by ashing in a muffle furnace at 550 ± 3°C. The ash residue was dissolved in 6 M HCl and quantitatively transferred to a 50 mL volumetric flask. Lanthanum nitrate and cesium chloride were added as matrix modifiers to ensure accurate measurements. Mineral concentrations were determined using a Varian Spectra AA 10 atomic absorption spectrometer (Varian Techtron Pty Limited, Mulgrave, Victoria, Australia) with an air-acetylene flame. Appropriate dilution factors were applied where required, and the results were expressed as mg/kg of lyophilized biomass.

Statistical analysis

The replicate cohort was considered the experimental unit, with three independent replicates per generation. Biological parameters are presented as mean ± standard deviation. Differences between the F3 and F4 generations were evaluated using a two-tailed Welch's t-test. The analyzed parameters included larval development duration, larval survival, peak average larval mass, adult lifespan, numbers of females and males, total egg production, and lifetime fecundity. All statistical analyses were performed in R version 4.5.1. employing the stats package for descriptive statistics and two-tailed Welch's t-tests, with statistical significance set at $\alpha = 0.05$.

Results and Discussion

Mineral composition

The concentrations of selected essential minerals and trace elements in the F3 sample of *S. littoralis* larval biomass (SLB) are presented in Table I. Among the analyzed elements, potassium (K) was present at the highest concentration, with an average value of 49,062.04 mg/kg, followed by magnesium (Mg) at 3,673.14 mg/kg, sodium (Na) at 2,126.11 mg/kg, and calcium (Ca) at 1,701.12 mg/kg. The concentrations of zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) were considerably lower, with mean values of 123.82, 39.73, 49.85, and 40.66 mg/kg, respectively. The results from the two measurements were generally consistent, indicating good agreement between replicates. According to Dobermann *et al*. (2017), *Tenebrio molitor* larvae contained approximately 828.28 mg/100 g potassium, 215.89 mg/100 g magnesium, 133.16 mg/100 g sodium, and 45.77 mg/100 g calcium on a dry matter basis. In comparison, the present *S. littoralis* sample contained substantially higher concentrations of these macrominerals, corresponding to 4,906.20 mg/100 g K, 367.31 mg/100 g Mg, 212.61 mg/100 g Na, and 170.11 mg/100 g Ca. Thus, potassium concentration in *S. littoralis* was approximately six times higher than that reported for *T. molitor*, while calcium, magnesium, and sodium concentrations were approximately 3.7, 1.7, and 1.6 times higher, respectively.

A similar comparison can be made for the trace elements. The zinc concentration in *S. littoralis* (12.38 mg/100 g) was comparable to that reported for *T. molitor* (12.53 mg/100 g), whereas copper and manganese concentrations were higher in *S. littoralis* (3.97 and 4.07 mg/100 g, respectively) than in *T. molitor* (1.62 and 1.14 mg/100 g). In contrast, the iron concentration of *S. littoralis* (4.99 mg/100 g) was slightly lower than the value reported for *T. molitor* (5.46 mg/100 g) (Dobermann *et al*., 2017). These differences indicate that *S. littoralis* biomass may represent a particularly rich source of several macro- and microminerals. It should be noted that direct comparisons among insect species should be interpreted with caution because mineral accumulation is influenced by species, developmental stage, diet, environmental conditions, and processing procedures.

Although Montalbán *et al*. (2023) did not determine the mineral composition of the resulting *T. molitor* biomass, the authors demonstrated that the type and proportion of agricultural by-products in the diet can significantly influence larval chemical composition. Their experimental diets, however, contained a standardized mineral and vitamin supplement, whereas *S. littoralis* larvae in the present study were reared exclusively on fresh broccoli florets without dietary supplementation. The potassium-dominated mineral profile observed here may therefore partly reflect the mineral characteristics of the broccoli substrate and the capacity of *S. littoralis* to retain or concentrate dietary minerals. Nevertheless, because the mineral composition of the original broccoli waste and the residual substrate was not determined, the extent of mineral transfer or concentration cannot be quantified. Future substrate-larva-frass mass-balance analyses would help distinguish dietary accumulation from species-specific mineral regulation and establish the efficiency with which individual elements are transferred into larval biomass.

Table I. Mineral composition of lyophilized *Spodoptera littoralis* larval biomass (SLB F3), expressed as mg/kg. Values represent two analytical determinations; the mean concentration and expanded measurement uncertainty are also shown.

Element	Sample weight (g)	Instrument reading (mg/L)	Final volume (mL)	Dilution factor	Individual result (mg/kg)	Mean (mg/kg)	Measurement uncertainty (mg/kg)
Zn	0.5114	1.25	50	1	122.21	123.82	19.08
	0.291	0.73	50	1	125.43		
Cu	0.5114	0.398	50	1	38.91	39.73	6.09
	0.291	0.236	50	1	40.55		
Fe	0.5114	0.51	50	1	49.86	49.85	7.69
	0.291	0.29	50	1	49.83		
Mg	0.5114	3.76	50	10	3676.18	3673.14	568.97
	0.291	2.136	50	10	3670.1		
K	0.5114	50.1	50	10	48983.18	49062.04	8055.99
	0.291	28.6	50	10	49140.89		
Na	0.5114	2.17	50	10	2121.63	2126.11	349.11
	0.291	1.24	50	10	2130.58		
Ca	0.5114	1.74	50	10	1701.21	1701.12	263.5
	0.291	0.99	50	10	1701.03		
Mn	0.5114	0.41	50	1	40.09	40.66	7.14
	0.291	0.24	50	1	41.24		

Proximate composition

The proximate composition of the F3 sample is presented in Table II. Protein was the predominant component, accounting for 48.04% of the sample. The fat content was 17.33%, while moisture and ash contents were 8.09% and 7.52%, respectively. Cellulose represented 6.50% of the analyzed material. Overall, the results indicate that the F3 biomass was characterized by a relatively high protein content, followed by fat as the second major component. According to Dobermann *et al*. (2017), *Tenebrio molitor* larvae contain approximately 48.35% protein and 38.51% fat on a dry matter basis. The protein content determined in the present study (48.04%) was therefore remarkably similarto that reported for *T. molitor*. In contrast, the fat content of F3 (17.33%) was considerably lower.

Comparison with *Bombyx mori* also shows interspecific differences in proximate composition. *B. mori* pupae have been reported to contain a higher protein content (61.8%) but substantially lower fat content (8.81%) than the SLB F3 sample. Thus, the protein content of F3 falls within the range reported for insect biomass, while its fat content is intermediate between the values reported for *T. molitor* and *B. mori* (Bednářová *et al*., 2013).
The cellulose content of the SLB F3 sample was 6.50%. Although direct comparison with published data is limited by differences in analytical methodology, previous studies have reported considerable amounts of structural fiber in edible insects. Bednářová *et al*. (2013) reported neutral detergent fiber (NDF), comprising cellulose, hemicellulose and lignin, at approximately 14% of dry matter in *Bombyx mori* pupae and 18% in *Tenebrio molitor* larvae. The cellulose content determined in the present study was therefore lower than the total fiber fraction reported for these species. This difference may be related to species-specific characteristics, developmental stage, diet and differences in analytical procedures. Nevertheless, the detection of 6.50% cellulose in *S. littoralis* biomass indicates the presence of a measurable structural carbohydrate fraction, which may contribute to its overall dietary fiber content.

A particularly relevant comparison can be made with the study by Montalbán *et al*. (2023), who reared *T. molitor* larvae on diets containing agricultural by-products, including broccoli by-product. Across the broccoli-based treatments, the mealworm larvae contained an average of 42.22% crude protein and 22.51% ether extract, whereas larvae receiving the diet with 100% broccoli by-product reached 60.09% protein and contained only 10.21% fat. The present *S. littoralis* biomass, with 48.04% protein and 17.33% fat, therefore occupied an intermediate position between these values. Despite differences in insect species, substrate preparation and experimental design, both studies indicate that broccoli-derived materials can support the production of protein-rich larval biomass and that increased protein deposition may be accompanied by a comparatively lower lipid content.

Importantly, Montalbán *et al*. (2023) also found that the nutritional composition of the larvae did not depend simply on the protein or fat concentration of the substrate. Both the type of by-product and its inclusion level affected larval composition, while diets consisting entirely of a single by-product reduced growth and diet digestibility. This distinction is relevant to the present study: the substantial protein content of *S. littoralis* suggests effective nutrient conversion from broccoli waste, although larval survival was suboptimal. Thus, broccoli waste appears suitable for generating nutrient-dense *S. littoralis* biomass, but supplementation or formulation of a more nutritionally balanced substrate may be required to improve biomass yield and larval survival.

Table II. Proximate composition of lyophilized *Spodoptera littoralis* larval biomass (F3). Values represent two analytical determinations and their mean, expressed as percentages.

			Average
Proteins	47.87	48.21	48.04
Lipids	17.25	17.41	17.33
Moisture	8.11	8.07	8.09
Ash	7.5	7.53	7.52
Celulose	6.58	6.41	6.5

Amino acid composition

The amino acid composition of the F3 biomass is presented in Table III. A total of 17 amino acids were analyzed, of which 16 were detected in the sample, while valine (Val) was not detected. The total content of detected amino acids was 35.84%. Among the analyzed amino acids, glutamic acid (Glu) was present at the highest concentration (6.70 ± 0.25%), followed by cysteine (Cys) (4.12 ± 0.03%) and aspartic acid (Asp) (3.33 ± 0.15%). Arginine (Arg) was also present at a relatively high concentration of 2.56 ± 0.06%, while leucine (Leu) and tyrosine (Tyr) were detected at concentrations of 2.26 ± 0.15% and 2.18 ± 0.09%, respectively. Alanine (Ala) and serine (Ser) were present at similar concentrations, with mean values of 1.93 ± 0.07% and 1.93 ± 0.08%, respectively.

Among the analyzed essential amino acids, leucine showed the highest concentration (2.26 ± 0.15%), followed by lysine (Lys) (1.67 ± 0.07%), threonine (Thr) (1.48 ± 0.12%), phenylalanine (Phe) (1.43 ± 0.02%), isoleucine (Ile) (1.42 ± 0.35%), methionine (Met) (0.80 ± 0.21%), and histidine (His) (0.73 ± 0.05%). Valine was not detected in the analyzed sample. Overall, the amino acid profile indicates the presence of several nutritionally important amino acids in the *S. littoralis* biomass, with glutamic acid representing the predominant amino acid among those analyzed.

The amino acid profile of the F3 biomass was compared with previously reported amino acid compositions of edible insects. A recent study by Oliveira *et al*. (2024) characterized the amino acid profile of *Tenebrio molitor* and *Gryllus assimilis* powders at different developmental stages and demonstrated that insect proteins contain substantial amounts of both essential and non-essential amino acids. In the present study, glutamic acid was the most abundant detected amino acid (6.70 g/100 g of sample), followed by cysteine (4.12 g/100 g) and aspartic acid (3.33 g/100 g). This pattern is generally consistent with the amino acid profiles reported for other insect species, in which glutamic and aspartic acids are commonly among the predominant amino acids.
Among the essential amino acids detected in F3, leucine showed the highest concentration (2.26 g/100 g), followed by lysine (1.67 g/100 g), threonine (1.48 g/100 g), phenylalanine (1.43 g/100 g), isoleucine (1.42 g/100 g), methionine (0.80 g/100 g), and histidine (0.73 g/100 g). In comparison, studies on *T. molitor* have also reported substantial levels of essential amino acids, particularly leucine, lysine, isoleucine and phenylalanine. Notably, lysine was identified as the limiting amino acid in *T. molitor* and *G. assimilis*(Oliveira *et al*., 2024).

The findings of Montalbán *et al*. (2023) also provide useful context for interpreting the amino acid composition of the F3 biomass. In their study, neither the inclusion level nor the type of agricultural by-product substantially altered the amino acid profile of *T. molitor* larvae, apart from comparatively small differences in alanine and proline. This relative stability contrasted with the pronounced substrate-related variation in crude protein and lipid contents, suggesting that insects may regulate the proportional composition of their proteins more strongly than the total quantities of protein and fat deposited. The broad range of amino acids detected in *S. littoralis*

reared exclusively on broccoli waste is consistent with this capacity to transform heterogeneous plant material into a more compositionally organized protein-containing biomass.

Direct numerical comparison nevertheless requires caution because Montalbán *et al*. (2023) expressed amino acids as percentages of crude protein, whereas the present results are expressed per 100 g of lyophilized biomass. When the present values are related to the measured crude protein content, glutamic acid represents approximately 13.95% of crude protein, which is close to the 12.02% reported for *T. molitor* larvae reared on broccoli-based diets. Aspartic acid accounted for approximately 6.93% of crude protein in F3 compared with 8.15% in the broccoli-fed mealworms. Conversely, the relative proportions of several indispensable amino acids, including leucine and lysine, were lower in F3. These differences may reflect species-specific protein composition, developmental stage, substrate characteristics and differences in hydrolysis and analytical procedures rather than substrate effects alone.

The absence of detectable valine should therefore be interpreted cautiously. Valine represented approximately 6.54% of crude protein in the broccoli-fed *T. molitor* larvae examined by Montalbán *et al*. (2023), and it is normally expected to occur in insect proteins. Its non-detection in the present sample should be regarded as an analytical result rather than evidence that *S. littoralis* biomass is biologically devoid of valine.

Table III. Amino acid composition of lyophilized *Spodoptera littoralis* larval biomass (SLB F3), expressed as g/100 g of sample.

	Amino acids	35.84
g/100 g of sample	Asp	3.33 ± 0.15
	Thr	1.48 ± 0.12
	Ser	1.93 ± 0.08
	Glu	6.70 ± 0.25
	Pro	1.48 ± 0.12
	Gly	1.82 ± 0.08
	Ala	1.93 ± 0.07
	Cys	4.12 ± 0.03
	Val	ND
	Met	0.80 ± 0.21
	Ile	1.42 ± 0.35
	Leu	2.26 ± 0.15
	Tyr	2.18 ± 0.09
	Phe	1.43 ± 0.02
	His	0.73 ± 0.05
	Lys	1.67 ± 0.07
	Arg	2.56 ± 0.06

Biological parameters

The biological parameters recorded for the two generations, designated here as F3 and F4, are presented in Table IV. The mean larval development duration was identical between generations (21.33 days), while larval survival was 29.67% in F3 and 28.67% in F4. The peak average larval mass was 0.89 ± 0.05 g in F3 and 0.90 ± 0.03 g in F4. Adult lifespan was also comparable between the two generations, with mean values of 10.33 days for both groups. The numbers of females and males were similar between generations, as was total egg

production. Lifetime fecundity was 1,046.44 ± 324.15 eggs per female in F3 and 1,033.33 ± 249.07 eggs per female in F4. No statistically significant differences were detected between the two generations for any of the analyzed biological parameters, including larval development duration, larval survival, peak average larval mass, adult lifespan, adult sex counts, total egg production, and lifetime fecundity (Welch's t-test, all $p > 0.56$).

Table IV. Biological performance of *Spodoptera littoralis* reared on broccoli waste over two consecutive laboratory generations.

Generation	Larval development duration (days)	Larval survival (%)	Peak average larval weight (g)	Adult lifespan (days)	Females (n)	Males (n)	Total egg production	Lifetime fecundity (eggs per female)
F3	21.33 ± 1.15	29.67 ± 4.04	0.89 ± 0.05	10.33 ± 2.31	14.67 ± 1.53	15.00 ± 2.65	15,043.33 ± 3,051.27	1,046.44 ± 324.15
F4	21.33 ± 0.58	28.67 ± 2.08	0.90 ± 0.03	10.33 ± 1.53	14.00 ± 1.00	14.67 ± 1.15	14,330.00 ± 2,558.69	1,033.33 ± 249.07

The biological performance recorded on broccoli florets can first be compared with previous results obtained from the same *S. littoralis* colony under alternative feeding regimes. Trajković *et al*. (2025) reported overall larval survival of 29.47% on a standard artificial diet, 42.47% on dandelion leaves and 35.73% on a beetroot-potato mixture over five consecutive generations. The survival recorded in the present study, 29.67% in F3 and 28.67% in F4, was therefore similar to that previously observed on the standard diet but lower than the values obtained on dandelion leaves and the beetroot-potato mixture. Larval development on broccoli florets lasted 21.33 days in both generations, compared with 16.36 days on dandelion leaves, 19.09 days on the beetroot-potato mixture and 19.57 days on the standard diet. Peak average larval mass on broccoli florets (0.89-0.90 g) was also somewhat lower than the final sixth-instar masses previously recorded on the standard, dandelion and beetroot-potato diets (1.04, 1.18 and 1.11 g, respectively). These comparisons suggest that broccoli florets sustained complete development and reproduction across two generations but were less favorable for larval survival and growth than the best-performing alternative diets. These dietary treatments represent different plant organs and should not be regarded as nutritionally equivalent merely because they are all fresh plant materials. The dandelion treatment consisted of leaves, while the beetroot–potato treatment consisted predominantly of storage roots and tubers. In contrast, the present study used reproductive inflorescence tissue and immature flower buds. Leaves, storage organs and inflorescences differ substantially in water content, structural carbohydrates, nutrient allocation and defensive metabolites, all of which may influence palatability, digestion and nutrient utilization by larvae. The observed differences in development and survival may therefore reflect not only plant species but also the plant organ supplied as food.

Hemmati *et al*. (2022) similarly demonstrated that larval development, survival, adult longevity and reproductive performance of *S. littoralis* depend strongly on the plant material consumed. Larvae reared on leaves of different legume cultivars required approximately 19.7-21.8 days to complete larval development, while adult female longevity ranged from approximately 7.1 to 10.0 days. The 21.33-day larval period and 10.33-day adult lifespan recorded on broccoli florets in the present study fall near or slightly above the upper ends of these ranges. However, Hemmati *et al*. used fresh legume leaves, and the experiments also differed in temperature, starting larval stage and rearing design. Consequently, the similarity in some life-history values should not be interpreted as evidence of equivalent dietary quality.

The findings of this study should be interpreted as preliminary evidence of the potential of broccoli-floret waste as a substrate for *S. littoralis* biomass production. Comparisons with published studies are necessarily limited by differences in insect species, developmental stages, rearing conditions, experimental duration, substrate

preparation, plant species and, importantly, the plant organ used. In addition, chemical characterization was based on duplicate rather than triplicate determinations, limiting the assessment of analytical variability. Although biological performance was evaluated using three independent cohorts per generation, only two consecutive generations were examined; this is insufficient to identify genuine generational trends, long-term adaptation or cumulative dietary effects. The absence of significant differences between the two generations should therefore be interpreted as short-term consistency under the tested conditions rather than evidence of generational stability. Accordingly, the present results constitute an exploratory proof of concept that establishes an initial nutritional and biological baseline. Further studies should include additional generations, larger numbers of independent cohorts, at least triplicate chemical determinations, direct characterization of the substrate and residual material, and controlled comparisons among different broccoli tissues and supplemented diets before firm conclusions regarding production efficiency, nutritional quality and broader applicability can be drawn.

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БИОКОНВЕРЗИЈА ОТПАДА ОД БРОКОЛИЈА ПОМОЋУ ВРСТЕ *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE)

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Извод

Све веће количине гомилања органског отпада и растући притисак на природне ресурсе указују на потребу за применом одрживих приступа искоришћавању ресурса. У том контексту, биоконверзија посредством инсеката представља перспективну стратегију за трансформацију органског отпада у биомасу богату хранљивим материјама. У овом истраживању испитиван је потенцијал памукове совице, *Spodoptera littoralis*, као биолошког агенса за производњу биомасе, при чему су коришћени цветови броколија из комуналног органског отпада као супстрат за узгој. Лиофилизована ларвена биомаса врсте *S. littoralis* (генерација F3) хемијски је окарактерисана одређивањем основног хемијског састава, минералног профила и аминокиселинског састава. Поред тога, током две генерације праћени су биолошки параметри како би се проценила уједначеност развоја ларви и репродуктивних перформанси у испитиваним условима узгоја. Биомаса је садржала 48,04% протеина и 17,33% липида, док су влага, пепео и целулоза чинили 8,09%, 7,52% и 6,50%, респективно. Међу анализираним минералним елементима, калијум је био најзаступљенији (49.062,04 mg/kg), а затим су следили магнезијум (3.673,14 mg/kg), натријум (2.126,11 mg/kg) и калцијум (1.701,12 mg/kg). Анализа аминокиселинског састава показала је укупни садржај детектованих аминокиселина од 35,84%, при чему је глутаминска киселина (6,70 ± 0,25%) била најзаступљенија, а затим су следили цистеин (4,12 ± 0,03%) и аспарагинска киселина (3,33 ± 0,15%). Међу детектованим есенцијалним аминокиселинама, леуцин је имао највећу концентрацију (2,26 ± 0,15%), док валин није детектован. Биолошки параметри били су упоредиви између две генерације, при чему нису утврђене статистички значајне разлике у трајању развоја ларви, преживљавању, маси ларви, животном веку одраслих јединки нити репродуктивним параметрима. Свеукупно, резултати показују да прах добијен лиофилизацијом јединки врсте *S. littoralis*, узгајаних на супстрату пореклом из отпада, садржи значајне количине протеина, липида, минералних материја и аминокиселина од нутритивног значаја. Ови налази указују на велики потенцијал примене познате пољопривредне штеточине у контролисаним системима биоконверзије и представљају основу за даља истраживања врсте *S. littoralis* као потенцијалне компоненте у стратегији циркуларне биоекономије.

Received: September 6th, 2026
Accepted: October 29th, 2026