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Feeding responses and digestive function of *Spodoptera littoralis* (Boisd) on various leafy vegetables exhibit possible tolerance traits

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Abstract

Spodoptera littoralis is a highly polyphagous pest that attacks numerous important crops in the world and causes substantial economic losses to agricultural production. In the present study, the effects of different leafy vegetables, including Purslane, Chives, Parsley, Basil, Dill, Coriander, and Mint, were investigated on feeding responses and enzymatic activities of *S. littoralis* under laboratory conditions. Furthermore, the total contents of the three major secondary metabolites (phenolics, anthocyanins, and flavonoids) in the studied vegetables were determined. Our findings showed that the lowest and the highest approximate digestibility were on Basil and Purslane, respectively. The highest values of efficiency of conversion of ingested and digested food were achieved in larvae fed on Chives and Coriander, respectively, whereas the lowest values were recorded after feeding on Purslane. The highest and lowest relative growth rates were in larvae reared on Dill and Purslane, respectively. Furthermore, the highest amylolytic and proteolytic activities were in larvae fed with Coriander and Dill, respectively, while the lowest activities of these enzymes were on Purslane. In addition, correlation analysis revealed significant correlations between feeding characteristics and enzymatic activity of *S. littoralis* with biochemical compounds of the studied leafy vegetables. Our results suggest that Coriander is a suitable host, while Purslane displayed tolerance traits against *S. littoralis*, which can be used in sustainable management programs aiming to reduce chemical inputs.

Introduction

Vegetables with edible leaves, known as leafy vegetables, are important fresh market crops worldwide and ranked fourth in terms of production after wheat, rice, and corn (Natesh *et al.*, 2017). Leafy vegetables are susceptible to numerous insect pests, in particular, *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae), also known as the Egyptian cotton leafworm (Sneh *et al.*, 1981; Khedr *et al.*, 2015; Hemmati *et al.*, 2022).

Spodoptera littoralis is a highly polyphagous pest with a wide host range covering at least 87 plant species and 40 plant families, including many vegetables, fruits, and ornamental plants (Ingram, 1975; Lanzoni *et al.*, 2012; Zamani Fard *et al.*, 2022). The management of *S. littoralis* is mostly carried out by using synthetic pesticides (Ismail, 2020). However, the extensive use of synthetic pesticides results in the development of resistant pests and imposes serious negative impacts on the environment. Therefore, it is necessary to develop alternative control measures for combating this pest (Azab *et al.*, 2001; Shishehbor and Hemmati, 2022).

Integrated pest management (IPM) is a sustainable pest control approach that uses a combination of management tools to successfully keep pest populations below harmful levels and minimize the reliance on pesticides (Thomas, 1999). Host plant resistance, which is defined as the use of pest-resistant crop varieties, is an effective, economical, and eco-friendly pest control method and is regarded as a fundamental component of IPM (Sharma and Ortiz, 2002; Golizadeh and Abedi, 2017).

Nutritional indices are valuable tools in recognizing the physiological and behavioral responses of insects toward host plants (Lazarevic and Peric-Mataruga, 2003; Golizadeh and Abedi, 2017; Babamir-Satehi *et al.*, 2022). Nutritional indices, especially efficiency of conversion of digested food (ECD), reflect the effects of phytochemicals on pest nutritional physiology (Hemati *et al.*, 2012*a*; Zamani Fard *et al.*, 2022).

The chemical composition of host plants influences the development, survival, and reproduction of insect pests (Awmack and Leather, 2002; Harvey *et al.*, 2007; Shishehbor and Hemmati, 2022). The secondary metabolites of plants, such as phenols and terpenes, may have toxic or anti-nutritional effects on insect herbivores (War *et al.*, 2011). Due to the negative effects of plants' secondary metabolites on insect pests, these compounds play a crucial role in increasing a host plant's tolerance against pests (Agrawal *et al.*, 1999). Investigating the digestive enzymes of *S. littoralis* and their nutritional indices on various leafy vegetables can be useful for selection of characteristics that develop insect pest-resistant plants (Zamani Fard *et al.*, 2022).

Feeding response and digestive physiology of *S. littoralis* are valuable variables for assessing the suitability or unsuitability of different host plants for this pest (Khedr *et al.*, 2015; Gacemi *et al.*, 2019; Shishehbor and Hemmati, 2022; Zamani Fard *et al.*, 2022). Previously, Khedr *et al.* (2015) examined the food consumption of *S. littoralis* on cotton genotypes and explored that Giza86 and Suvin were unsuitable varieties for this pest. Furthermore, Shishehbor and Hemmati (2022) investigated the growth rate and nutritional performance of *S. littoralis* on 11 bean cultivars and reported that the common bean Arabi and cowpea Mashhad are suitable and unsuitable hosts for this pest, respectively. Zamani Fard *et al.* (2022) demonstrated that among various mung bean cultivars, Simite 1 and Simite 2 were less suitable for *S. littoralis*, based on feeding efficiency and digestive enzyme activities. Biological and population growth parameters of *S. littoralis* on different leafy vegetables were demonstrated by Hosseini Mousavi *et al.* (2022), who reported that Coriander was the relatively susceptible host for *S. littoralis*, while Purslane was identified as relatively resistant.

To the best of our knowledge, previous studies have not investigated the nutritional physiology of *S. littoralis* on various leafy vegetables. The economic damage of *S. littoralis* on vegetables, as well as the harmful residual effects of the chemical pesticides used against this pest on the vegetables, makes this topic even more essential for research. Therefore, this research aimed to evaluate the effect of various leafy vegetables on feeding responses and the digestive enzyme activities of *S. littoralis*, including proteases and amylases. Furthermore, the relationships between growth properties and enzymatic activity of this pest with secondary plant metabolites of leafy vegetables were investigated. Our results could be useful in developing new approaches for the management of *S. littoralis*, including the use of resistant vegetables against *S. littoralis* that aim to diminish chemical involvement.

Materials and methods

Plant cultivation

The seeds of various leafy vegetables, including Purslane (*Portulaca oleracea* L.), Chives (*Allium schoenoprasum* L.), Parsley (*Petroselinum crispum* (Mill.)), Basil (*Ocimum basilicum* L.), Dill (*Anethum graveolens* L.), Coriander (*Coriandrum sativum* L.), and Mint (*Mentha spicata* L.) were obtained from the Seed and Plant Improvement Institute of Karaj, Iran and planted in the research farm of the Faculty of Agriculture at Shahid Chamran University of Ahvaz, Iran. After the emergence, the leaves of the studied leafy vegetables were transferred to a growth chamber (25 ± 1°C, relative humidity of 65 ± 5%, and a 16:8 h light: dark photoperiod). The leaves of vegetables were used at the end of the vegetative growth stage for *S. littoralis* feeding.

Spodoptera littoralis *colony*

The larvae of *S. littoralis* were collected from various fields at the Safiabad Agricultural Research and Training Center and Natural Resources in Dezful, Iran. Leafy vegetables were used to rear the initial larval colonies under controlled conditions of 25 ± 1°C, relative humidity of 65 ± 5%, and a 16:8 h light: dark photoperiod in a growth chamber (Shishehbor and Hemmati, 2022). Adults of *S. littoralis* were fed with a honey solution (10%). Before experiments, *S. littoralis* were reared on each leafy vegetable for two generations. After that, the third-generation colony was used for investigating the feeding performance and digestive enzyme activities of *S. littoralis* on the studied leafy vegetables.

Nutritional indices of S. littoralis

To quantify of the nutritional indices of *S. littoralis*, 40 neonate larvae were considered for each leafy vegetable. The larvae of *S. littoralis* were reared together in plastic containers (15 cm diameter × 25 cm height) until the third instar emerged. Then, the third instar larvae were individually (25 replicates for each cultivar) transferred into Petri dishes (8 × 1 cm) to prevent any cannibalistic behavior. The weight of third to sixth instar larvae, food consumed, and frass produced were recorded daily before and after feeding on various leafy vegetables until the feeding stopped and reached the pre-pupal stage. Since similar trends were found for each leafy vegetable during each instar, data are presented to encompass this entire period. Furthermore, for determining the percentage of the dry weight of *S. littoralis* larvae, food and frass produced, 25 samples were weighed for each tested leafy vegetable, dried in an oven at 60°C for 48 h, and then weighed again. Nutritional indices of *S. littoralis* larvae, including consumption index (CI), approximate digestibility (AD), the efficiency of conversion of ingested food (ECI), the ECD, relative consumption rate (RCR), and relative growth rate (RGR) were evaluated by Waldbauer (1968) formulas. Moreover, the weight of the pre-pupal and pupal stages of *S. littoralis* were assessed 24 h after their appearance on each tested leafy vegetable based on the dry weight.

Quantification of enzymatic activities in S. littoralis *larvae*

Preparation of larval midgut extract

Sixth instar *S. littoralis* larvae were anesthetized on ice and dissected under a stereomicroscope. The midguts of 50 larvae of *S. littoralis* were homogenized on ice and prepared as described by Hemmati *et al.* (2017). Homogenates were centrifuged at 15,000 × g at 4°C for 10 min, and then supernatants were collected and stored at −20°C for enzymatic assays. All assays (each tested leafy vegetable) were carried out in three replicates (Hemati *et al.*, 2012*b*).

Quantification of proteolytic and amylolytic activities

Proteolytic activity was determined utilizing azocasein (1.5%) as substrate in the universal buffer system (50 mM sodium phosphate-borate) at pH 11. The reaction mixture containing 50 μl of the midgut extract and 80 μl of the substrate in 50 mM universal buffer was incubated at 37°C for 50 min. Proteolysis was stopped by adding 100 μl of 30% trichloroacetic acid (TCA), followed by cooling at 4°C for 30 min and centrifugation at 14,000 × *g* for 10 min. An equal volume of 2 M NaOH was added to the supernatant, and the absorbance was measured at 440 nm (Elpidina *et al.*, 2001). Moreover, the amylase activity of *S. littoralis* larvae fed with different leafy vegetables was examined using starch 1% as a substrate in the universal buffer system (10 mM succinate-glycine-2, morpholinoethan sulfonic acid) at pH 10. The mixtures containing midgut extracts and 1% starch were incubated at 37°C for 30 min. The enzymatic reaction was stopped by adding 50 μl of DNSA reagent and heating in boiling water for 15 min. The adsorption of mixture was read at 540 nm after cooling on ice (Bernfeld, 1955).

Secondary metabolites analysis of leafy vegetables

Biochemical characteristics of various leafy vegetables, including total phenolics, flavonoids, and anthocyanins, were studied to discover the relationship between the phytochemical levels and nutritional physiology of *S. littoralis*. Experiments were carried out in three replicates for each tested vegetable.

Preparation of plant extract

One gram of wet leafy vegetables was weighed, crushed, and transferred to an ice pack. After that, 10 ml of 80% methanol was gradually added to the contents of the mortar until a uniform solution was obtained. After passing through Whatman No. 1 filter paper, the obtained solution was transferred into a 1.5 ml vial. Finally, the solid and liquid phases in the extract were separated using a centrifuge at 15,000 × g for 5 min.

Quantification of total phenolics, flavonoids, and anthocyanins contents of leafy vegetables

The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu reagent according to the method described by Slinkard and Singleton (1997). The absorbance was determined at 765 nm utilizing gallic acid as a standard compound. Furthermore, the quantity of anthocyanins in the leafy vegetable extracts was measured according to Kim *et al.* (2003) method. The absorbance of the mixture was read at 520 nm using cyanidin as a standard. Moreover, the flavonoid content of the studied plant extracts was measured using aluminum chloride colorimetric as described by Zhishen *et al.* (1999). The absorbance was read at 430 nm using catechin as a standard.

Statistical analysis

After testing for normality using the Shapiro-Wilk test, all data gotten from determining nutritional indices, digestive enzyme activities, and the content of phytochemicals were analyzed by one-way multivariate analysis of variance (MANOVA) by SPSS ver 22. Tukey's HSD test was used to compare the statistical differences among means at a $P < 0.01$ level. The dendrogram of various leafy vegetables was created based on all the tested parameters of *S. littoralis* by applying Ward's minimum-variance hierarchical clustering method utilizing SPSS ver 22. Furthermore, correlation analysis between the nutritional indices and enzyme activities of *S. littoralis* with biochemical properties of various leafy vegetables were explored using Pearson's correlation test using SPSS ver 22.

Results

Feeding responses of S. littoralis

The feeding performance of the third to sixth instar larvae of *S. littoralis* reared on various leafy vegetables is indicated in table 1. The nutritional indices of *S. littoralis* were significantly different on the tested leafy vegetables. The larvae fed with Purslane host (5.91) had the highest CI value, and the lowest value was observed when the larvae were fed with Chives (0.630) ($F_{6,\ 168} = 126.82$; $P < 0.01$). The highest AD value of the larvae was on Purslane (90.511%), and the lowest was on Basil (30.414%) ($F_{6,\ 168} = 299.90$; $P < 0.0001$). The highest values of ECI were recorded on Chives (42.946%), while the lowest one was observed on Purslane (12.166%) ($F_{6,\ 168} = 146.44$; $P < 0.01$). The maximum value of ECD was found on Coriander (65.451%), and the minimum value was obtained on the Purslane (13.494%) ($F_{6,\ 168} = 90.91$; $P < 0.0001$). Among the tested leafy vegetables, Purslane (0.451 mg mg^{-1} d^{-1}) had the highest RCR value ($F_{6,\ 168} = 126.82$; $P < 0.0001$), and the lowest one was on Coriander (0.234 mg mg^{-1} d^{-1}). The *S. littoralis* larvae reared on Dill (0.085 mg mg^{-1} d^{-1}) had the highest value of RGR index, and the lowest value was observed on Purslane (0.053 mg mg^{-1} d^{-1}) ($F_{6,\ 168} = 17.62$; $P < 0.0001$) (table 1).

The results in fig. 1 showed that the highest value of the whole larval instars weight was detected on Basil (52.59 mg), and the lowest one was recorded on Chives (37.55 mg) ($F_{6,\ 168} = 64.01$; $P < 0.01$) (fig. 1a). The lowest food consumed was achieved by feeding larvae on Chives (23.52 mg) ($F_{6,\ 168} = 170.20$; $P < 0.0001$) (fig. 1b). In contrast, the larvae of *S. littoralis* reared on the Basil (17.01 mg) and Dill (16.92 mg) indicated as the maximum value of larval gain weight ($F_{6,\ 168} = 28.19$; $P < 0.0001$) (fig. 1c). The highest value of frass produced came from larvae fed on Basil (49.55 mg), and the lowest one was on Chives (5.178 mg) and Purslane (8.478 mg) ($F_{6,\ 168} = 258.65$; $P < 0.0001$) (fig. 1d).

Moreover, significant differences in the pre-pupal and pupal weights were shown in the cotton leafworms fed on various leafy vegetables (fig. 2). The *S. littoralis* fed on Coriander revealed the heaviest pre-pupal ($F_{6,\ 168} = 49.47$; $P < 0.0001$) and pupal ($F_{6,\ 168} = 17.56$; $P < 0.0001$) weights (fig. 2a, b).

Digestive enzyme activity of S. littoralis

The results of amylolytic and proteolytic activities of *S. littoralis* larvae fed on various leafy vegetables explored that the enzyme

Table1. Nutritional indices (mean ± SE) of the third to sixth instar of *Spodoptera littoralis* reared on various leafy vegetables

Vegetable	CI	AD (%)	ECI (%)	ECD (%)	RCR (mg mg^{-1} d^{-1})	RGR (mg mg^{-1} d^{-1})
Coriander	0.938 ± 0.025e	42.045 ± 1.491d	35.691 ± 1.216b	65.451 ± 1.865a	0.234 ± 0.006e	0.083 ± 0.003ab
Basil	1.362 ± 0.036c	30.414 ± 1.338e	23.977 ± 0.624d	55.302 ± 1.794b	0.340 ± 0.009c	0.081 ± 0.002ab
Dill	1.216 ± 0.032d	43.310 ± 1.485d	28.183 ± 0.621c	52.818 ± 1.450b	0.304 ± 0.008d	0.085 ± 0.002a
Chives	0.630 ± 0.020f	77.830 ± 0.778b	42.946 ± 1.272a	55.481 ± 1.993b	0.157 ± 0.005f	0.066 ± 0.001c
Mint	1.519 ± 0.038b	59.229 ± 0.941c	19.487 ± 0.466e	33.219 ± 1.149c	0.379 ± 0.009b	0.073 ± 0.001bc
Parsley	1.245 ± 0.037cd	62.564 ± 1.537c	24.116 ± 0.471d	39.491 ± 1.754c	0.311 ± 0.009cd	0.074 ± 0.002ab
Purslane	1.804 ± 0.041a	90.511 ± 0.662a	12.166 ± 0.812f	13.494 ± 0.923d	0.451 ± 0.010a	0.053 ± 0.002d

CI, consumption index; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate.
The means followed by different letters in the same column are significantly different (Tukey test, $P < 0.01$).

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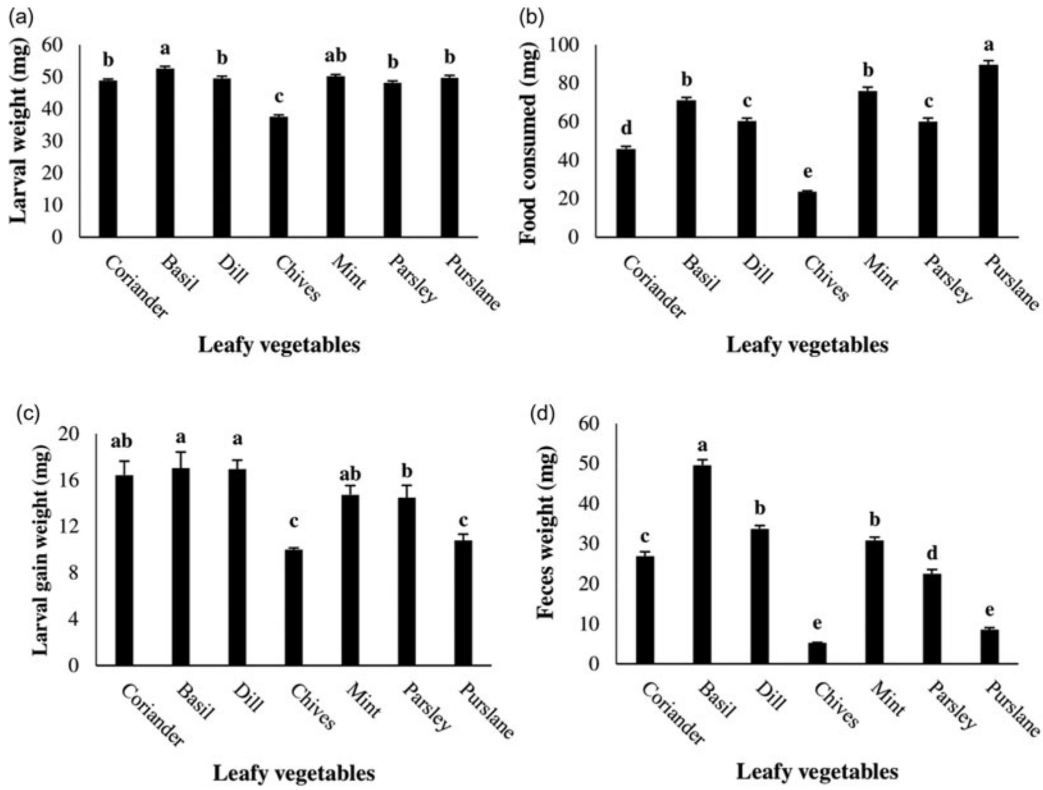

Figure 1. (a) Mean larval weight, (b) food consumed, (c) larval gain weight and (d) feces produced of *Spodoptera littoralis* reared on various leafy vegetables.

activities were significantly different in the studied hosts (fig. 3). The highest specific amylase activity was obtained with larvae fed on Coriander (0.734 mU mg^{-1}) and Basil (0.657 mU mg^{-1}), while the lowest amount was found on Mint (0.166 mU mg^{-1}), Parsley (0.232 mU mg^{-1}) and Purslane (0.137 mU mg^{-1}) ($F_{6,\ 14} = 45.71$; $P < 0.0001$) (fig. 3a). Furthermore, the highest total proteolytic activity was significantly associated with larvae reared on Dill (2.394 mU mg^{-1}), and the lowest one was related to larvae fed on Purslane (0.829 mU mg^{-1}) ($F_{6,\ 14} = 18.39$; $P < 0.0001$) (fig. 3b).

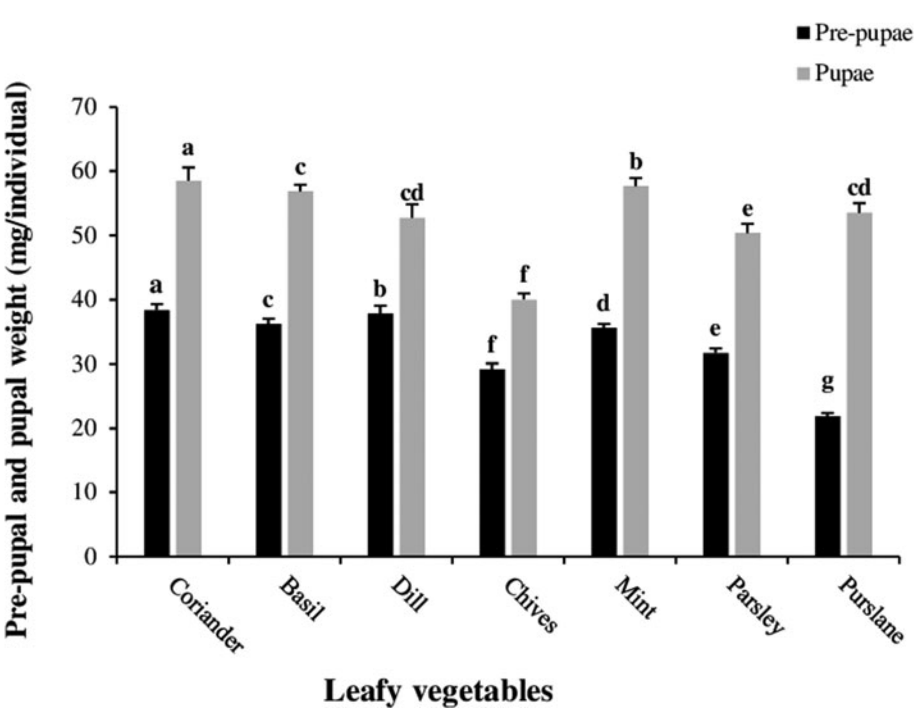

Figure 2. Pre-pupal and pupal weight (mg) of *Spodoptera littoralis* reared on various leafy vegetables.

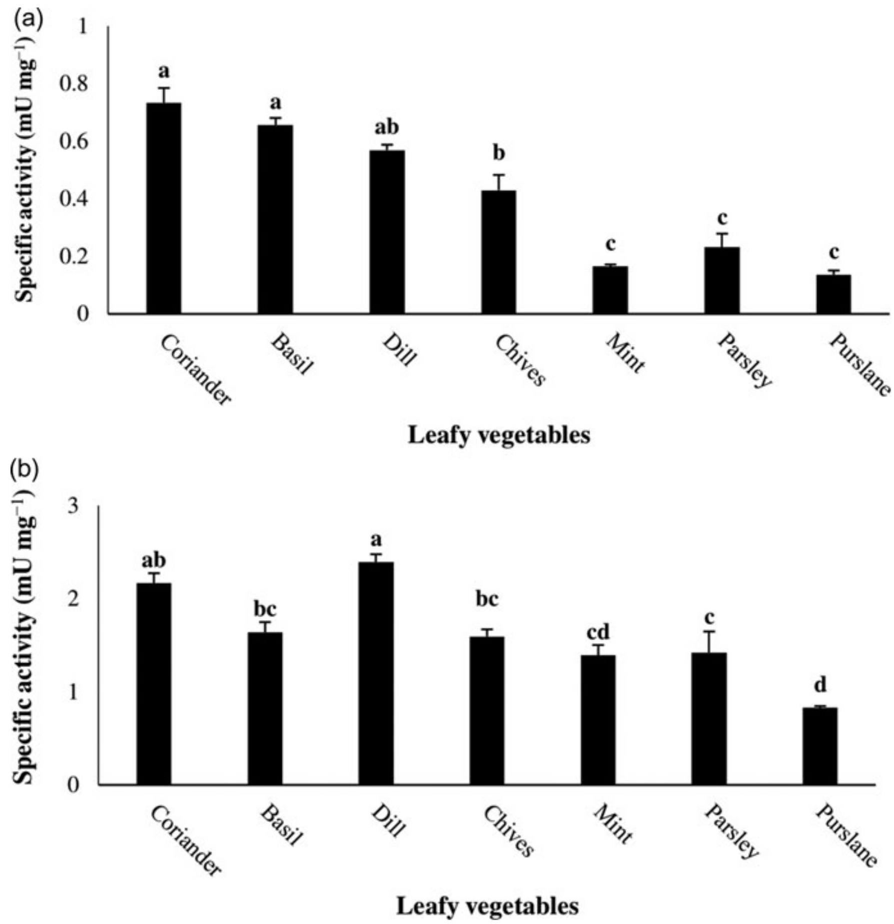

Figure 3. Amylolytic (a) and general proteolytic (b) activity of midgut extracts from sixth instar larvae of *Spodoptera littoralis* reared on various leafy vegetables.

Cluster analysis

The dendrogram based on feeding responses and enzymatic activities of *S. littoralis* on various leafy vegetables is shown in fig. 4. Two clusters including A and B, are apparent in the dendrogram. Sub-cluster A_1 includes Mint, Parsley, and Chives, and sub-cluster A_2 comprises Purslane. Sub-cluster B_1 includes Basil and Dill, and sub-cluster B_2 contains Coriander (fig. 4).

Secondary metabolites content in various leafy vegetables

The findings indicated that the concentrations of secondary metabolites in various leafy vegetables were significantly different (table 2). Purslane (14.123 mg ml^{-1}) and Coriander (129.68 mg ml^{-1}) had the lowest and highest total phenols contents, respectively ($F_{6,\ 14} = 16{,}360$; $P < 0.0001$). Chives (119.00 mg ml^{-1}) and Purslane (331.223 mg ml^{-1}) had the lowest and highest quantities

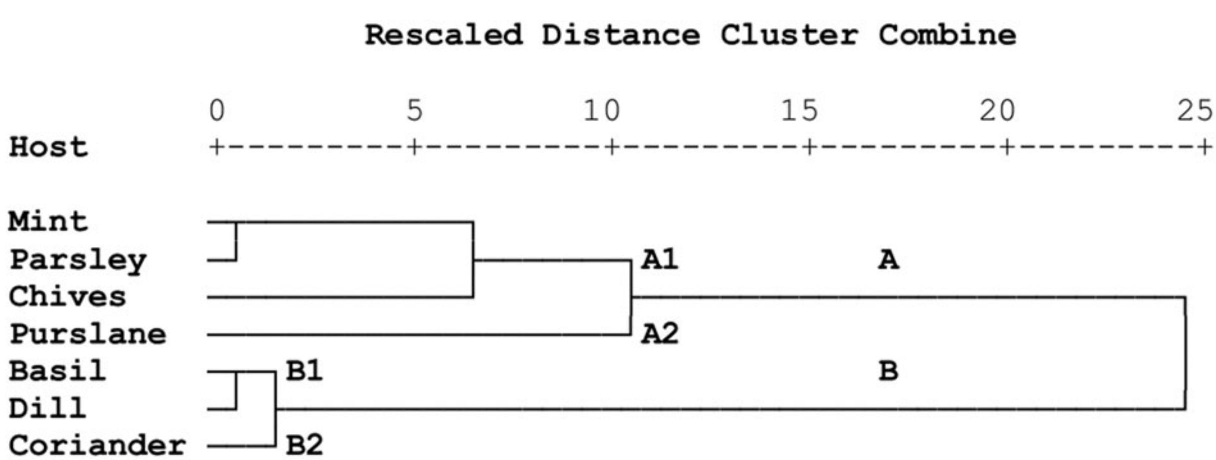

Figure 4. Dendrogram of various leafy vegetables based on nutritional indices and enzymatic activities of *Spodoptera littoralis* reared on various leafy vegetables (Ward's method).

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Table 2. Biochemical characteristics (mean ± SE) (mg ml^{-1}) of various leafy vegetables

Vegetable	Total phenolic content	Flavonoids content	Anthocyanins content
Coriander	129.68 ± 0.018a	186.770 ± 0.011e	0.497 ± 0.000a
Basil	107.57 ± 0.036c	253.440 ± 0.023c	0.159 ± 0.000c
Dill	57.766 ± 0.088e	176.770 ± 0.011f	0.338 ± 0.001b
Chives	16.070 ± 0.011f	119.000 ± 0.288g	0.050 ± 0.001d
Mint	88.743 ± 0.020d	194.546 ± 0.026d	0.341 ± 0.001b
Parsley	126.51 ± 0.008b	290.116 ± 0.017b	0.500 ± 0.011a
Purslane	14.123 ± 0.014g	331.223 ± 0.014a	0.058 ± 0.000d

The means followed by different letters in the same column are significantly different (Tukey test, $P < 0.01$).

of flavonoids, respectively ($F_{6,\ 14} = 44{,}033$; $P < 0.0001$). In addition, the highest amount of anthocyanins was obtained for Parsley (0.500 mg ml^{-1}) and Coriander (0.497 mg ml^{-1}), while the lowest amount was detected for Chives (0.050 mg ml^{-1}) and Purslane (0.058 mg m^{-1}) ($F_{6,\ 14} = 1858.34$; $P < 0.0001$) (table 2).

Correlation analysis

Analysis of Pearson's correlation coefficients of nutritional performances and growth of *S. littoralis* with their enzymatic activity when fed on various leafy vegetables are presented in table 3. Significant correlations were detected between the feeding and growth of *S. littoralis* and the amylolytic and proteolytic enzyme activity on the various leafy vegetables. The food consumed and RCR of *S. littoralis* showed significant negative correlations with amylolytic and proteolytic activities ($P < 0.05$). In contrast, the proteolytic and amylolytic activities of larvae were positively correlated with ECI, ECD, and RGR indices of *S. littoralis* ($P < 0.05$). Furthermore, there was no significant correlation between larval and pupal weights of the Egyptian cotton leafworm with the enzyme activities of larvae ($P > 0.05$) (table 3).

In addition, the results of correlation analysis between nutritional and physiological characteristics of *S. littoralis* with biochemical traits of the tested leafy vegetables are indicated in table 4. Larval and pupal weight, ECD, and RGR indices of *S. littoralis* revealed significant positive correlations with the total phenolic content of tested leafy vegetables. In contrast, the ECI and ECD indices and proteolytic and amylolytic activities of larvae were negatively correlated with the flavonoid content of various leafy vegetables ($P < 0.05$). There was no significant correlation between all nutritional and physiological traits, except the RGR index, and proteolytic activity of *S. littoralis* with the anthocyanin amounts of various leafy vegetables ($P > 0.05$). Furthermore, a significant positive correlation was detected between flavonoid content of various leafy vegetables and larval weight, food consumed, and RCR index ($P < 0.05$) (table 4).

Table 3. Pearson's correlation coefficients (*r*) between nutritional indices and digestive enzyme activity of *Spodoptera littoralis* larvae on various leafy vegetables

Parameter	Amylolytic activity	Proteolytic activity
Larval weight	0.063 (0.785)	−0.026 (0.911)
Food consumed	−0.440 (0.046)	−0.486 (0.026)
Pupal weight	0.155 (0.501)	0.116 (0.617)
ECI	0.585 (0.005)	0.561 (0.008)
ECD	0.942 (0.000)	0.764 (0.000)
RCR	−0.523 (0.015)	−0.541 (0.011)
RGR	0.668 (0.001)	0.706 (0.000)

ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate. The numerals in the parenthesis are *P* value.

Table 4. Pearson's correlation coefficients (*r*) between nutritional and physiological characteristics of *Spodoptera littoralis* with biochemical traits of various leafy vegetables

Parameter	Total phenolic	Flavonoids	Anthocyanin
Larval weight	0.495 (0.022)	0.578 (0.006)	0.338 (0.134)
Food consumed	0.022 (0.926)	0.776 (0.000)	−0.063 (0.786)
Pupal weight	0.552 (0.010)	0.337 (0.135)	0.431 (0.051)
ECI	0.027 (0.909)	−0.821 (0.000)	0.075 (0.746)
ECD	0.486 (0.026)	−0.481 (0.027)	0.277 (0.224)
RCR	−0.060 (0.797)	0.784 (0.000)	−0.110 (0.635)
RGR	0.626 (0.002)	−0.372 (0.097)	0.587 (0.005)
Proteolytic activity	0.329 (0.145)	−0.598 (0.004)	0.460 (0.036)
Amylolytic activity	0.351 (0.118)	−0.438 (0.047)	0.177 (0.442)

ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate. The numerals in the parenthesis are *P* value.

Discussion

Our results showed that feeding performance, as well as proteolytic and amylolytic activities of *S. littoralis* were significantly affected by various leafy vegetables. These results are consistent with the previous studies related to *S. littoralis* fed on various host plants (Ladhari *et al*., 2013; Khedr *et al*., 2015; Khafagi *et al*., 2016; Gacemi *et al*., 219; Shishehbor and Hemmati, 2022; Zamani Fard *et al*., 2022; Hemmati *et al*., 2022).

The AD index indicates the degree of food that is digestible and absorbable through the midgut wall (Hemati *et al*., 2012*a*). According to our results, the highest amount of AD index and food consumed were observed in larvae fed with Purslane. Our findings revealed that the larvae feeding on Purslane digested most of the food consumed, while *S. littoralis* larvae were unable to use the digested substance to gain body weight. A reason for the high amount of food consumption and AD on Purslane may be due to its defense quality, e.g., low concentration of two secondary metabolites, including phenols and anthocyanins content compared with other leafy vegetables (Shishehbor and Hemmati, 2022). Another reason is related to that *S. littoralis* consumes large amounts of Purslane to compensate for restricted access to nutrients (Hemmati *et al*., 2021). The highest consumption of Purslane by *S. littoralis*, combined with the lowest of the larval frass, resulted in the highest value of AD on this leafy

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vegetable. Conversely, the lowest AD index of *S. littoralis* larvae was associated with Basil, while the highest values of larval weight, larval weight gain, and frass produced were obtained with the same vegetable. The observed difference in the AD index depends on the physical and chemical characteristics of the host plant (Gacemi *et al.*, 2019). It has been revealed that the AD and RCR indexes of insect pests are almost high in the undesirable host plants, while the RGR, ECD, and ECI indexes are low on the same plants (Biggs and Mcgregor, 1996), which was similar to our findings on Purslane.

ECI is one of the most important nutritional indices of polyphagous insects for determining food quality, which measures an insect's ability to use food for growth and development (Batista Pereira *et al.*, 2002; Hemati *et al.*, 2012*a*). According to the results, the lowest ECI value of *S. littoralis* larvae fed on Purslane can be attributed to low ability to convert ingested food into body biomass (Babamir-Satehi *et al.*, 2022). According to correlation analysis, ECI and ECD values of *S. littoralis* were negatively correlated with the flavonoid content of the leafy vegetables. It explores that a higher amount of the flavonoid content in Purslane may be an important reason for the lower ECI and ECD values of *S. littoralis* on this leafy vegetable. The lowest ECI and ECD indices of the larvae fed on Purslane suggest an antibiotic mechanism and, consequently, an unsuitable quality of this host for the development of *S. littoralis* (Scriber and Slansky, 1981; Zamani Fard *et al.*, 2022).

The highest value of the RGR index of larvae on Dill and Coriander indicates the high efficiency of *S. littoralis* larvae in converting ingested food into body mass, which can reveal the high nutritional value of these vegetables for this pest. Furthermore, the lowest RGR value obtained in the larvae fed on Purslane might contribute to plant secondary chemical compounds, such as high total flavonoid content, which has been previously reported (War *et al.*, 2011; Shishehbor and Hemmati, 2022; Babamir-Satehi *et al.*, 2022; Zamani Fard *et al.*, 2022; Hemmati *et al.*, 2022). Secondary metabolites in plant tissues interfere with the production of nutrients needed for the growth of the insect pest (Price *et al.*, 1980; Harvey, 2005). Moreover, the lowest value of the RGR in the Purslane is associated with the lowest value of the ECI on this leafy vegetable. In addition, larvae with a lower RGR index retard their development due to maximizing the AD to achieve food requirements (Barton Browne and Raubenheimer, 2003).

ECD indicates the part of the digested food that is converted into the insect's body tissues, and therefore the changes in the ECD are accompanied by a relative increase or decrease in the digestion of metabolized food for energy (Hemati *et al.*, 2012*a*). According to the results, the highest value of ECD in *S. littoralis* larvae on Coriander signifies a higher efficiency in utilizing metabolized food to produce body tissues. Furthermore, the lowest value of ECD on Purslane indicates the low quality of this vegetable for the development of *S. littoralis*. The highest value of AD beside the lowest value of RGR and larval frass on Purslane suggests the high cost of digestion and the low amount of food absorption by *S. littoralis* on this vegetable. Also, the prolongation of the larval stage of *S. littoralis* on Purslane significantly reduced the efficiency of conversion of ingested and digested food into larval body tissues. Studies have shown that digestive enzyme activity is a crucial factor that affects the efficiency of converting digested food into larval body biomass (Lazarevic and Peric-Mataruga, 2003; Zamani Fard *et al.*, 2022). In other words, the effect of phytochemicals on the digestive enzymes activities of the insect pest is inferred from the ECD index (Babamir-Satehi *et al.*, 2022). Therefore, the lowest ECD of *S. littoralis* on Purslane is likely due to digestive enzyme inhibitors or secondary chemical compounds in this vegetable that adversely affect the food digestion by the larvae, which could explain the unsuitability of this leafy vegetable for *S. littoralis*. The findings of correlation analysis revealed that the ECD value of *S. littoralis* was positively correlated with the digestive enzyme activities of this pest on various leafy vegetables. It suggests that the lowest proteolytic and amylolytic activities in Purslane may explain the lowest value of ECD of *S. littoralis* on this vegetable. The inactivation of digestive enzymes by inhibitors leads to poor nutrient utilization, growth retardation, starvation of insects, and their death (Hemmati *et al.*, 2021). Overall, the highest values of CI and RCR, and the lowest total proteolytic activity in larvae fed with Purslane indicate the low quality of this vegetable for *S. littoralis* development.

In the present study, there were significant differences in the proteolytic and amylolytic activities of the midgut extract of *S. littoralis* larvae among various leafy vegetables. The findings demonstrated that the highest total proteolytic activity occurred in the larvae reared on Dill and Coriander, which is probably due to the high protein content of these vegetables. According to the ECI, ECD, and RGR values, the larvae reared on Dill and Coriander had the highest capacity to convert the ingested food into biomass, confirming the high quality of these vegetables for feeding and growth of *S. littoralis*. Furthermore, the lowest amylolytic activity of *S. littoralis* on Purslane could be related to the presence of amylase inhibitors or low carbohydrate content in this host plant (Franco *et al.*, 2002). In the present study, a significant correlation was found between the total flavonoid contents and digestive enzyme activities of *S. littoralis* larvae. The relationship between digestive enzyme activities and the composition of various host plants suggests the adaptive nature of *S. littoralis*, which can detect the content of the consumed food and regulate the levels of enzyme activities (Kotkar *et al.*, 2009).

In this research, cluster analysis exposed that the studied leafy vegetables could be divided into four distinct sub-clusters (A1, A2, B1, and B2), relying on the nutritional indices and enzymatic activity of *S. littoralis*. The clustering might be due to a high level of biochemical similarity among leafy vegetables. Sub-cluster A_1 comprised Mint, Parsley, and Chives as relatively unsuitable hosts, and sub-cluster A_2 included Purslane as the most unsuitable vegetable for *S. littoralis*. Moreover, sub-cluster B_1 consisted of Basil and Dill as relatively suitable vegetables, and sub-cluster B_2 included Coriander as the most suitable host for *S. littoralis* feeding. Overall, the unsuitability of the Purslane could be a result of the lower nutritional value based on the results of the feeding responses and digestive enzyme activities obtained in *S. littoralis* larvae on this leafy vegetable.

In conclusion, our findings reveal that *S. littoralis* can complete development on various leafy vegetables; but not all vegetables seem to be similarly suitable for the growth and feeding of this pest. Coriander is the most suitable host due to the ideal growth of *S. littoralis*, suggesting this vegetable is a good source of nutrition for this pest. However, the growth rate was the lowest in *S. littoralis* larvae fed with Purslane, probably due to the high concentration of secondary biochemical metabolites, especially the total flavonoids. The present results and those of Hosseini Mousavi *et al.* (2022) suggest that Purslane, as an unsuitable host, contains some plant inhibitors that mediate antibiosis to insect pests reflected by the weak performance of *S. littoralis* on this vegetable. Further research is required to examine the potential of amylase and protease inhibitors on the level of enzymatic activities of *S. littoralis*, as a key factor in the susceptibility or resistance of leafy vegetables toward this pest. Identification of these features enables us to explain differential levels of tolerance to *S. littoralis* among the leafy vegetables, which could help develop transgenic plants resistant to *S. littoralis*.

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Conflict of interest. The authors declare that they have no conflict of interests.

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