

# Effects of cabbage cultivars on the food consumption and utilization parameters of diamondback moth, *Plutella xylostella* (L.)

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#### Abstract

Food consumption and utilization parameters of *Plutella xylostella* (L.) were studied on the leaves of eight cabbage cultivars under laboratory conditions  $(27 \pm 2 \,^{\circ}\text{C}, 70 \pm 5 \,^{\circ}\text{R}$  RH, 16 L: 8D photoperiod). The present study evaluated the effect of different biochemical factors of cabbage cultivars on the food consumption and utilization parameters of *P. xylostella*. All the food consumption rate (RCR) and relative growth rate (RGR) were observed on the cabbage cultivars 'NS 183', 'DEB 806', 'Green Express' and 'Mohor'. The highest values of the efficiency of conversion of ingested (3.94 %) and digested (4.95 %) food was observed on the cabbage cultivar 'DEB 806'. In contrast, the 4th instar *P. xylostella* larvae fed on the cabbage cultivar 'Lucky Ball' exhibited the lowest values of RCR (1.31 mg food consumption and utilization parameters of *P. xylostella* weight gain/mg body weight/ day), ECI (2.47 %) and ECD (2.95 %). The food consumption and utilization parameters of *P. xylostella* showed a non-significant correlation with the total glucosinolate content and total phenolic content of cabbage cultivars. The present study will help to identify effective resistant or tolerant cabbage cultivars for the development of Integrated Pest Management strategies against *P. xylostella*.

Keywords Host plant resistance · Assimilation · Secondary metabolites · Nutrients

## Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the important cruciferous vegetable crops grown in India. India is the second highest producer of cabbage in the world with an annual production of 9369 thousand tonnes and productivity of 23.25 metric tonnes ha<sup>-1</sup> from an area of 403 thousand hectares during the year 2019-20 (Anonymous 2020). The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is the most devastating pest of cabbage causing upto 50–80% annual loss in marketable yield (Devjani and

Singh 1999; Ayalew 2006) and annual losses of about US \$ 16.0 million in India (Mohan and Gujar 2003). The outbreak of this pest is due to its wide geographical distribution, ability to migrate over long distances, high reproductive potential, tendency to acquire insecticide resistance and the elimination of its natural enemies due to the widespread use of broad-spectrum insecticides (Gryzwacz 2010; Canico 2013; Furlong 2013). *P. xylostella* has gained multiple resistance to major insecticide groups including the new generation insecticides and *Bacillus thuringiensis* Berliner (*Bt*) formulations under field conditions (Sarfraz and Keddie 2005; Oliveira et al. 2011; Neto et al. 2016; Zhang et al. 2016). This situation has prompted the necessity to formulate a sustainable and eco-friendly management strategy for *P. xylostella*, capable of minimizing pre-harvest losses in cabbage.

Host Plant Resistance is an integral component of Integrated Pest Management (IPM) and can effectively reduce insect pest infestation (Stout 2014). The host plant biochemicals influence food consumption and utilization by the insect herbivore; thereby, affecting its growth and development (Behmer 2009; Santolamazza-Carbone et al. 2016; Wetzel et al. 2016; Nouri-Ganbalani et al. 2018). The generalist

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insects usually depend on nutrients such as proteins, carbohydrates and other primary metabolites for their feeding activity on plants (Ratzka et al. 2002). In addition to these nutritional factors, the feeding activity of the crucifer specialist insects is stimulated by specific chemical cues known as glucosinolates (Renwick and Lopez 1999). Glucosinolates are naturally occurring secondary metabolites found mostly in the plants of the family Brassicaceae (Santolamazza-Carbone et al. 2016). Other secondary plant metabolites like phenols, abundant in brassicaceous plants (Cartea et al. 2011), can negatively affect the nutritional physiology of insects and reduce its performance (War et al. 2018).

Food consumption and utilization parameters generate valuable information about an insect's relative rate of food intake and its physiological capacity to assimilate the ingested and digested food for growth and development (Waldbauer 1968). These parameters also link the nutritional performance of an insect herbivore with various host plant attributes (Scriber and Slansky 1981; Slansky 1992; Wetzel et al. 2016). Numerous studies have assessed the effects of plant biochemical factors on nutritional physiology of insects (Senthil-Nathan 2013; Robin et al. 2017; Nouri-Ganbalani et al. 2018). Despite the economic importance of *P. xylostella*, no information is available on how the food consumption and utilization parameters of this pest are influenced by the different biochemical factors of cabbage. Therefore, the present study aimed to investigate the effect of biochemical factors of cabbage on the food consumption and utilization parameters of P. xylostella. This study would provide further insight into the nutritional physiology of the insect-host plant interactions and could help to design a comprehensive Integrated Pest Management strategy against P. xylostella on cabbage.

## **Materials and methods**

#### **Raising of cabbage cultivars**

Seedlings of eight cabbage commercial cultivars (Table 1) were raised under the insect-proof nets at Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur. Thirty days old cabbage seedlings were transplanted in separate plots measuring  $3.0 \times 3.0$  m with a spacing of 60 cm between rows and 40 cm between plants. Fertilizers at 120:60:40 kg/ha (N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O) were incorporated into the soil before transplanting. Plants were maintained free of any pests and no chemical pesticides were used.

# Insect culture

The culture of field-collected *P. xylostella* larvae were maintained on fresh leaves of each cabbage cultivar for multiple generations in glass jars (15 cm diameter and 20 cm depth) 
Table 1
Details of cabbage cultivars used for the experiment on food consumption and utilization parameters of *P. xylostella*

Cabbage cultivars	Source		
Lucky Ball	Kaneko Seeds Co., Ltd.		
Rare Ball	Kaneko Seeds Co., Ltd.		
Saurabh	Namdhari Seeds Pvt. Ltd.		
Blue Jays	Tokita Seed India Pvt. Ltd.		
NS 183	Namdhari Seeds Pvt. Ltd.		
DEB 806	Debgiri Agro Products Private Ltd.		
Mohor	Bharat Nursery Pvt. Ltd.		
Green Express	Acsen HyVeg Pvt. Ltd.		

at a temperature of  $27 \pm 2$  °C,  $70 \pm 5\%$  relative humidity and 16 L: 8D photoperiod. The leaves (food) were changed daily until the feeding ceased in the pre-pupal stage. Newly emerged moths were released in separate glass jars of the same size as above with excised leaves of each cabbage cultivar for mating and oviposition. A cotton ball soaked in 10% honey diet (1 part of honey: 9 parts of water) was kept in each glass jar as food for the adult moths. The leaves of cabbage cultivars with newly laid eggs were carefully put inside the separate glass jars for hatching. The newly moulted 4th instar *P. xylostella* larvae were utilized for the experiments.

# Food consumption and utilization parameters

The food consumption and utilization parameters of 4th instar P. xylostella larvae were studied on the leaves of eight-week-old plants of the selected cabbage cultivars under laboratory conditions at a temperature of  $27 \pm 2$  °C,  $70 \pm 5\%$  relative humidity and 16 L: 8D photoperiod. The fresh leaves of each cabbage cultivar were pre-weighed and placed in individual plastic vials (40 mm diameter x 60 mm height) with perforated lids. Newly moulted 4th instar larvae were obtained from the insect culture and were initially pre-starved for 6 h. The larvae were weighed and released individually in each plastic vial with fresh cabbage leaves. Each cabbage cultivar was replicated five times with ten vials per replication. After two days of feeding, the weight of the 4th instar P. xylostella larvae, unconsumed leaves of each cabbage cultivar (food) and faeces were weighed. Simultaneously, a control with the same amount of fresh leaves without larvae was maintained for each cabbage cultivar under similar conditions to determine the natural weight loss.

The food consumption and utilization parameters of *P. xylostella* were worked out for each cabbage cultivar using the standard formulae as described by Waldbauer (1968):

Relative consumption rate (RCR) (mg food consumed/mg body weight/day) =  $Q/(S \times T)$ .

Efficiency of conversion of ingested food (ECI) (%) =  $R/Q \ge 100$ .

Efficiency of conversion of digested food (ECD) (%) =  $R/(Q-U) \ge 100$ .

where, Q is corrected fresh weight of leaves consumed (mg), R is the weight gained by larva (mg), S is mean weight of larva (mg) during the feeding period, U is weight of faeces produced (mg) in each treatment, T is feeding time (in days). The corrected fresh weight of leaves consumed by larva during the feeding period due to natural loss of moisture was calculated using the formula: Q = [1-a/2][W-(L+bL)], where Q is corrected fresh weight (mg), W is weight of food given (mg), L is weight of uneaten food (mg), a is the ratio of moisture loss to the initial weight and b is the ratio of moisture loss to the final weight.

#### Extraction and assay of biochemical constituents

Standard protocols were used to assess the biochemical constituents in the leaves of each cabbage cultivar. Before the start of the experiment, fresh leaf samples from eightweek-old cabbage plants were oven-dried at 60 °C for 36 h. Total phenol content in leaves (mg gallic acid equivalent/g dry weight) was determined using the Folin & Ciocalteu's reagent (Sigma-Aldrich, India) method (Vinson et al. 1998). The total protein and carbohydrate content of each cabbage cultivar was estimated using the method of Sadasivam and Manickam (2007). The total protein and carbohydrate content of each cabbage cultivar was expressed as a ratio of protein: carbohydrate (P:C).

The total glucosinolate content in cabbage leaves ( $\mu$ moles/g dry weight) was calculated from the sinigrin hydrate (Sigma-Aldrich, India) analytical standard (McGhee et al. 1965). The enzyme myrosinase catalyzes the conversion of glucosinolates into unstable isothiocyanates in brassicaceous plants (Ratzka et al. 2002; Wallace and Eigenbrode 2002). To deactivate this myrosinase enzyme, 0.3 g of ovendried cabbage leaf sample was extracted by boiling at 80 °C for 15 min with 0.3 ml of 60% methanol (Merck, India). Four ml of 0.2 mM sodium (II) tetrapalladate (Sigma-Aldrich, India) was added to the supernatant (40 µl) obtained after centrifugation of the extracted sample.

#### **Data analysis**

Data on food consumption and utilization parameters were used to compute the analysis of variance (ANOVA) for testing the significance of differences among the cabbage cultivars using completely randomized design (CRD). A Tukey's Honestly Significant Difference test at a 5 per cent probability was used to compare the significance of differences among the cabbage cultivars. Before statistical analyses, the percentage data on ECI and ECD were subjected to arcsine transformation (Gomez and Gomez 1984). The correlation of different biochemical constituents in leaves of cabbage cultivars with the food consumption and utilization parameters was also worked out. The data analysis was carried out with the software IBM SPSS version 23.0 for Windows (IBM Corporation, Armonk, New York, USA).

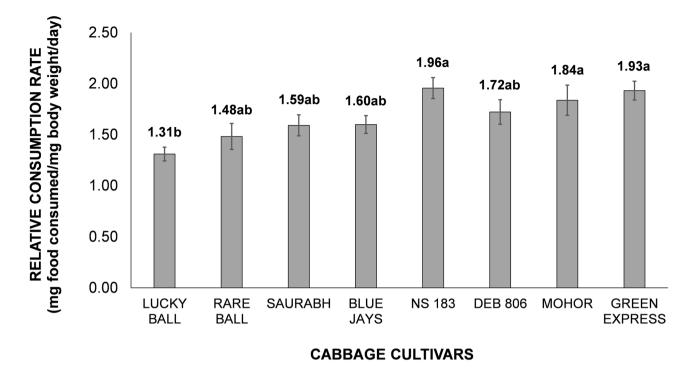
#### **Results and discussion**

#### Food consumption and utilization parameters

The results on the food consumption and utilization parameters of 4th instar P. xylostella larvae on different cabbage cultivars are presented and discussed herein. Food consumption and utilization parameters viz.. RCR, RGR, ECI and ECD of P. xylostella differed significantly among all the eight cabbage cultivars tested in the present study. Relative consumption rate (RCR) indicating the relative rate of food intake by 4th instar P. xylostella larvae was significantly higher on the cabbage cultivars 'NS 183', 'Green Express' and 'Mohor' (Fig. 1; F = 4.33; df = 7, 32; p = 0.002). The lowest values of relative consumption rate were recorded on the cabbage cultivar 'Lucky Ball'. Relative growth rate (RGR) was highest for *P. xylostella* larvae fed on the leaves of the cabbage cultivars 'DEB 806', 'NS 183', and 'Green Express' (Fig. 2; F = 10.42; df = 7, 32; p < 0.0001). Significantly lower values of RGR was recorded on the cabbage cultivars 'Lucky Ball'. The highest and lowest values for Efficiency of conversion of ingested food (ECI) (Fig. 3; F=20.39; df=7, 32; p<0.0001) and Efficiency of conversion of digested food (ECD) (Fig. 4; F = 54.64; df = 7, 32; p < 0.0001) were recorded on the cultivars 'DEB 806' and 'Lucky Ball', respectively.

Food consumption and utilization parameters are appropriate indices to assess the nutritional physiology of insect herbivores on host plants (Hemati et al. 2012). The results of the present investigation showed a significant decline in the RCR values of 4th instar *P. xylostella* larvae when fed on the leaves of the cabbage cultivar 'Lucky Ball'. Lower consumption rates of *P. xylostella* might be due to the differences in the nutritional quality of food or presence of secondary plant metabolites (Scriber and Slansky 1981; Hariprasad and Emden 2010). Lower RCR was associated with a decrease in faeces production which might be due to prolonged retention of the ingested food in the larval digestive tract (Scriber and Slansky 1981).

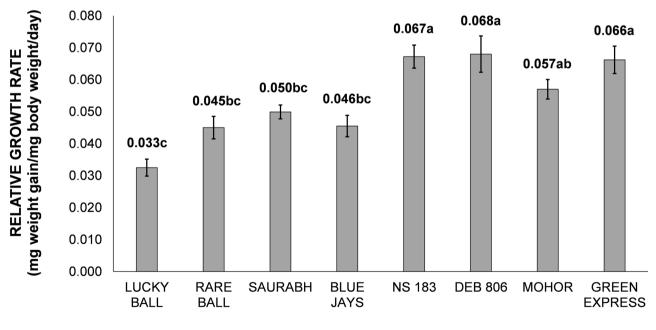
The efficiency with which the ingested and digested food is assimilated for growth and development determines the



**Fig. 1** Relative consumption rate (mg food consumed/mg body weight/day) of 4th instar *P. xylostella* larvae on eight cabbage cultivars; Means above bars followed by different letters are significantly different ( $p \le 0.05$ )

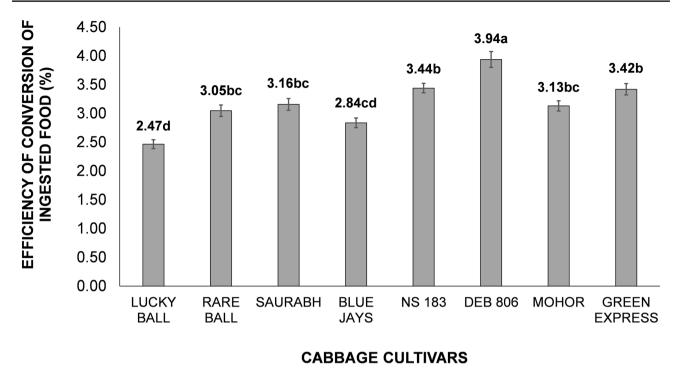
degree of food utilization by the insect herbivore (Namin et al. 2014). ECI and ECD indicate the physiological efficiency of an insect to assimilate the ingested and digested

food for its growth and development (Hemati et al. 2012). The reduced RGR of *P. xylostella* larvae on the cabbage cultivars 'Lucky Ball' and 'Blue Jays' was apparently due to a



**CABBAGE CULTIVARS** 

**Fig. 2** Relative growth rate (mg weight gain/mg body weight/day) of 4th instar *P. xylostella* larvae on eight cabbage cultivars Means above bars followed by different letters are significantly different ( $p \le 0.05$ )



**Fig.3** Efficiency of conversion of ingested food (%) of 4th instar *P. xylostella* larvae on eight cabbage cultivars; Means above bars followed by different letters are significantly different ( $p \le 0.05$ )

decrease in ECI and ECD values. The *P. xylostella* larvae fed on these cabbage cultivars were least efficient at assimilating the ingested and digested food for growth and development as indicated by the low ECI and ECD values. A significant decline in ECI and ECD values of *P. xylostella* larvae might be due to the metabolic costs associated with the biosynthesis and maintenance of detoxification enzymes for plant secondary metabolites in the larval gut (Ratzka et al. 2002). The present results on food consumption and utilization parameters of *P. xylostella* are in agreement with the findings of Kaur (2001) and Nouri-Ganbalani et al. (2018) on *Brassica oleracea var. botrytis* L. and *Brassica napus* L., respectively.

#### Glucosinolates

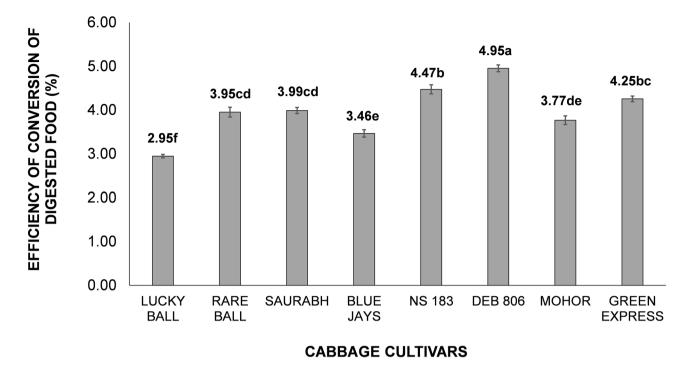
The present results revealed a significant variation in the total glucosinolate content of leaves among the tested cabbage cultivars (Fig. 5; F = 16.52; df = 7, 32; p < 0.0001). The total glucosinolate content was significantly higher in the leaves of cabbage cultivar 'Green Express' and 'Blue Jays'. The cultivars 'Rare Ball', 'Mohor' and 'NS 183' exhibited significantly lower values for total glucosinolate content. The present results are in close agreement with Kusznierewicz et al. (2008) and Poelman et al. (2008), who found that the total glucosinolate content in cabbage cultivars ranged from 3.3 to 7.7  $\mu$ moles of total glucosinolate/g dry weight and 0.67 to 8.91  $\mu$ moles of total glucosinolate/g dry weight, respectively.

#### **Total phenols**

The cabbage cultivars differed statistically for the total phenol content in leaves (Fig. 6; F=3.58; df=7, 32; p=0.006). The highest and lowest values for total phenol content were recorded from the leaves of the cultivars 'Rare Ball' and 'Mohor', respectively. Previous studies by Kamath et al. (2015), Karoui et al. (2018) and Kusznierewicz et al. (2008) also showed that the total phenol content of cabbage cultivars varied from 0.81 to 4.9 mg gallic acid equivalent/g dry weight of leaf tissue, which supports the present findings.

#### Protein: Carbohydrate (P:C) ratio

The P:C ratio in cabbage leaves differed statistically among the cabbage cultivars (Fig. 7; F=3.04; df=7, 32; p=0.014). The highest and lowest values for P:C ratio were recorded from the leaves of the cultivars 'DEB 806' and 'Lucky Ball', respectively. The present results are in close agreement with the findings of Adelanwa and Medugu (2015).



**Fig. 4** Efficiency of conversion of digested food (%) of 4th instar *P. xylostella* larvae on eight cabbage cultivars; Means above bars followed by different letters are significantly different ( $p \le 0.05$ )

# **Correlation studies**

Correlation of different biochemical constituents in leaves of cabbage cultivars with food consumption and utilization parameters of *P. xylostella* was worked out and presented in Table 2. The results showed a positive and non-significant correlation of the total glucosinolate content with RCR (r=0.248; p=0.554), RGR (r=0.315; p=0.447), ECI (r=0.265; p=0.527) and ECD (r=0.190; p=0.652) of *P. xylostella* (Table 2). Total phenol content registered a negative,

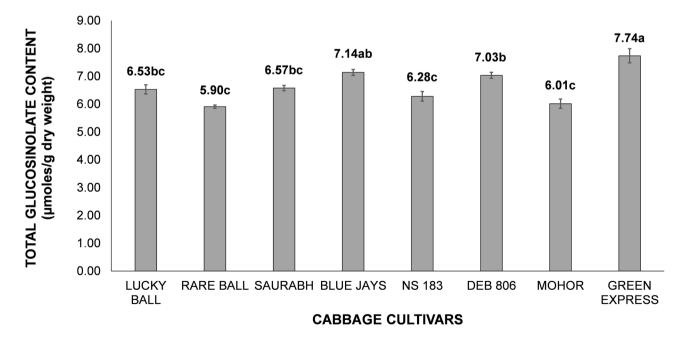
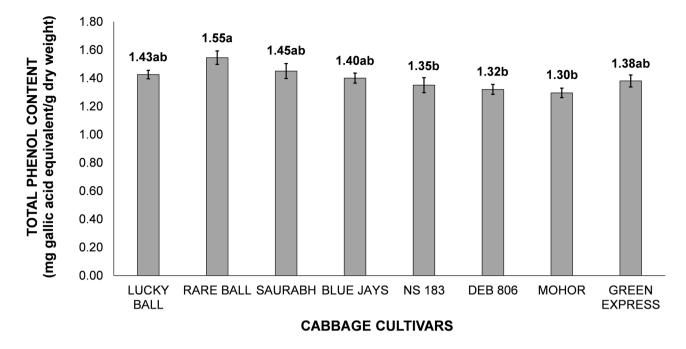


Fig. 5 Total glucosinolate content ( $\mu$ moles/g dry weight) in leaves of eight-week old cabbage cultivars; Means above bars followed by different letters are significantly different ( $p \le 0.05$ )

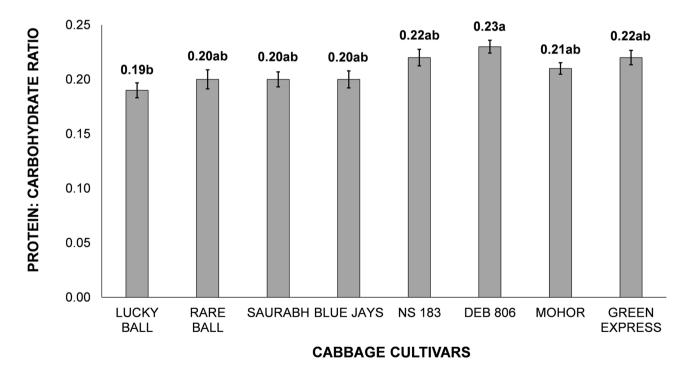


**Fig. 6** Total phenol content (mg gallic acid equivalent/g dry weight) in leaves of eight-week old cabbage cultivars; Means above bars followed by different letters are significantly different ( $p \le 0.05$ )

non- significant correlation with RCR (r=-0.658; p=0.076), RGR (r=-0.631; p=0.093), ECI (r=-0.469; p=0.241) and ECD (r=-0.353; p=0.392) of *P. xylostella* (Table 2). Highly significant and positive correlation was recorded between the protein: carbohydrate (P:C) ratio in cabbage leaves and

RCR (r=0.799; p=0.017), RGR (r=0.966; p<0.0001), ECI (r=0.946; p<0.0001) and ECD (r=0.910; p=0.002) of *P. xylostella* (Table 2).

Correlation studies showed a positive but non-significant correlation of the total glucosinolate content with relative



**Fig. 7** Protein: carbohydrate (P:C) ratio in leaves of eight-week old cabbage cultivars; Means above bars followed by different letters are significantly different ( $p \le 0.05$ )

Experimental parameters		Statistical parameters	Total glu- cosinolate	Total phenol	Protein: Carbohydrate ratio
Relative consumption rate (RCR)	Correlation Coefficient		0.248	-0.658	$0.799^{*}$
	p value		0.554	0.076	0.017
Relative growth rate (RGR)	Correlation Coefficient		0.315	-0.631	0.966**
	p value		0.447	0.093	< 0.0001
Efficiency of conversion of ingested food (ECI)	Correlation Coefficient		0.265	-0.469	0.946**
	p value		0.527	0.241	< 0.0001
Efficiency of conversion of digested food (ECD)	Correlation Coefficient		0.190	-0.353	$0.910^{**}$
	p value		0.652	0.392	0.002

Table 2 Correlation of different biochemical factors of cabbage cultivars with food consumption and utilization parameters of P. xylostella

Significant at  $p \le 0.05$  and  $p \ge 0.01$  level of probability, respectively

consumption rates (RCR) of P. xylostella on the tested cabbage cultivars. Previous authors also found no direct association between total glucosinolate content of cabbage cultivars and larval feeding rates of *P. xylostella* (Bodnaryk 1992; Poelman et al. 2008; Robin et al. 2017). The present results conform to the findings of Arany et al. (2008) and Bodnaryk (1997), who also reported a non-significant association of total glucosinolate content with larval feeding rates of P. xylostella on Arabidopsis thaliana L. and Brassica juncea L. The total glucosinolate content of cabbage cultivars did not affect the relative growth rates and food utilization parameters of P. xylostella larvae in the present investigation. Our results are in agreement with previous authors who also found no correlation between total glucosinolate content and larval performance of crucifer specialist feeders like cabbage stem flea beetle (Doring and Ulber 2020), stem weevil (Eickermann et al. 2011) and root fly (Birch et al. 1992). Blau (1978) and Li et al. (2000) reported that artificial diets with a higher concentration of allyl glucosinolates did not affect the performance of Pieris rapae L. and P. xylostella larvae, which supports the present findings.

Glucosinolates are secondary metabolites containing non-volatile nitrogen and sulphur-linked glycosides and are known to occur in plants belonging to the family Brassicaceae (Charron and Sams 2004). The enzyme myrosinase catalyzes the conversion of glucosinolates into toxic isothiocyanates in brassicaceous plants (Ratzka et al. 2002; Wallace and Eigenbrode 2002). These toxic isothiocyanates usually act as feeding deterrents and inhibit growth rates in generalist insects (Hopkins et al. 2009; Santolamazza-Carbone et al. 2016). However, specialist crucifer feeders have evolved different detoxification strategies to counteract myrosinase-isothiocyanate toxicity (Jeschke et al. 2016). P. xylostella can avoid this myrosinase hydrolysis by employing a desulfation strategy and minimize the post-ingestion toxicity of glucosinolates (Heidel-Fischer et al. 2019). The ingested glucosinolates are rapidly desulfated into non-toxic desulfo-glucosinolates by glucosinolate-sulfatase (GSS) enzyme in the gut lumen. These non-toxic desulfoglucosinolates no longer act as substrates for myrosinase and are excreted out with faecal matter (Ratzka et al. 2002).

The present investigation showed a negative association of total phenolic content with all the food consumption and utilization parameters of *P. xylostella*. Numerous studies have highlighted the deleterious effects of plant phenolics in insects as feeding deterrents and anti-digestion factors (Appel 1993; Bennett and Wallsgrove 1994; Resse 1978). Plant phenols impair insect gut metabolism and prevent nutrient uptake by covalently binding to dietary proteins, lipids and digestive enzymes (Felton et al. 1992). These compounds also inhibit insect growth through oxidative stress by the generation of oxygen and phenoxy radicals (War et al. 2012).

In addition to these secondary metabolites, nutrients such as proteins, carbohydrates play an essential role in determining the total food intake and its utilization by the insect herbivore; thereby influencing its growth and development (Scriber and Slansky 1981; Slansky 1993). In the present study, the food consumption and utilization parameters of P. xylostella showed a highly significant, positive correlation with the protein: carbohydrate (P:C) ratio in leaves of cabbage cultivars. Dietary protein is an important limiting factor than carbohydrate for insect growth (Scriber and Slansky 1981; Wilson et al. 2019), as plant tissue generally have a low concentration of usable nitrogen and essential amino acids (Felton 1996). Insects fed on imbalanced food (low protein: carbohydrate ratio) compensate for the low nutritional value of food by an increase in its feeding rates (Lee et al. 2004; Slansky 1993). A compensatory increase in relative consumption rates was also exhibited by P. xylostella for low P:C ratio on all the cabbage cultivars in the present investigation. Such compensation in feeding rates might have increased the ingestion of plant secondary metabolites by P. xylostella on cabbage cultivars (Bernays 1990). The metabolic costs incurred for the detoxification of these secondary metabolites possibly resulted in low RGR, ECI and ECD values on cabbage cultivars.

# Conclusions

The food consumption and utilization parameters of 4<sup>th</sup> instar P. xylostella larvae on the cabbage cultivar 'Lucky Ball' was characterized by significantly lower rates of food intake and reduced efficiency to assimilate the ingested and digested food for growth and development. The present findings suggest that the cabbage cultivar 'Lucky Ball' was not suitable as host for food consumption and its utilization by the 4th instar P. xylostella larvae. The total glucosinolate content and total phenol content of cabbage cultivars did not affect the food consumption and utilization parameters of P. xylostella. The food consumption and utilization parameters of *P. xylostella* were positively correlated to the protein: carbohydrate (P:C) ratio of cabbage cultivars. The present study helps to identify effective resistant or tolerant cabbage cultivars for the development of Integrated Pest Management strategies against P. xylostella. Further studies are required to ascertain the role of specific plant metabolites on the food consumption and utilization parameters of P. xylostella.

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#### Declarations

**Conflict of interest** The authors declare they have no conflict of interest.

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