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# Nutritional indices of Helicoverpa armigera (Lep.: Noctuidae) on seeds of five host plants

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### Abstract

In this study, the nutritional indices of *Helicoverpa. armigera* larvae on five different host plants including cowpea, chickpea, soybean, navy bean and corn were determined in the laboratory. The lowest relative consumption rate (RCR) of the third instar larvae was on cowpea  $(3.21\pm0.512)$ . The efficiency of conversion of ingested food (ECI) and the relative growth rate (RGR) were the highest on cowpea  $(6.8\pm0.004 \text{ and } 0.243\pm0.007, \text{ respectively})$ . The lowest values of efficiency of conversion of ingested food (ECI) and Efficiency of conversion of digested food (ECD) ( $2.4\pm0.002$  and  $6.9\pm0.005$ , respectively) of the fourth instar larvae were observed on corn. The highest ECI and ECD of the fifth instar larvae ( $23.2\pm0.010$  and  $84.5\pm0.10$ , respectively) and the sixth instar larvae ( $26.7\pm0.017$  and  $40.5\pm0.011$ , respectively) were obtained on cowpea. The Approximate digestibility (AD) of whole larval instars (the third to sixth instars) on different host plants ranged from  $28.9\pm0.024$  to  $55\pm0.015$ , which was the lowest on corn and highest on cowpea. The larvae fed on cowpea showed the highest values of ECI and ECD ( $20.1\pm0.002$  and  $83.4\pm0.080$ , respectively) and RGR ( $7.59\pm0.15$ ). **Key words:** Nutritional indices, *Helicoverpa armigera*, host plants.

## Helicoverpa armigera (Lep.: Noctuidae)

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#### Introduction

The cotton bollworm, Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae), is one of the important pests of various agricultural crops with global distribution. This pest is a typical polyphagous species and attacks more than 200 crop species including soybean (Fathipour and Naseri, 2011), cotton, chickpea, pigeonpea, tobacco, maize and a range of vegetables and fruit crops (Fitt, 1989). Wide host range, multiple generations, migratory behavior and high fecundity enable H. armigera to survive in unstable habitats and become a difficult pest to control (Subramanian and Mohankumar, 2006). The use of chemical insecticides has traditionally been the primary management option for H. armigera control but their continuous use has led occurring resistance in this pest (Gunning et al., 1996). Therefore, it is necessary to devise a suite of environmentally safe pest management tactics to control the damage caused by this pest. The use of resistant host plants has been considered an important component of any integrate pest management (IPM) program. Identification of host plant resistance mechanisms enable us to select resistant genotypes in order to use in plant breeding programs (Kranthi et al., 2002).

Polyphagous insects feed on different host plants in which the chemical composition of these plants significantly affects their population density. Therefore, the quality and quantity of food utilization directly affect growth, development, and reproduction of insects (Scriber and Slansky, 1981). An insect's performance on a given diet is reflected by its ability to digest, convert the diet into biomass and the insect's relative growth rate (Price *et al.*, 1980). Therefore, nutritional indices are considered as important factors determining resistance in host plants to insects. Also, analyses of these parameters reveal how food digest and convert into body mass (Dettner and Prutz, 2005) and will help us manage *H. armigera* populations.

Ashfaq *et al.* (2003) investigated on morpho-physical factors affecting consumption and coefficient of utilization of *H. armigera*. Some studies have been carried out on the effects of different varieties of soybean on nutritional indices of *H. armigera* (Naseri *et al.*, 2009; Soleimannejad *et al.*, 2010). The nutritional indices of this pest has been studies on different varieties of tomato (Srinivasan and Uthamasamy,

2005) but no study measured and compared the nutritional indices of this pest on different species of host plants.

In this study, the effect of the seeds of five different host plants (cowpea, chickpea, soybean, navy bean and corn) on nutritional indices of *H. armigera* were studied in the laboratory. In natural conditions, most life stages of this pest feed on seeds of the mentioned host plants, therefore measuring and comparing the nutritional indices of *H. armigera* on seeds of different commercial host plants may help us to evaluate relative antibiotic resistance of these plants to this pest. Also, these indices can help us increase our knowledge on the insect-plant interactions.

### **Materials and Methods**

**Plant seeds:** Seeds of five different host plants including cowpea ('Mashhad'), chickpea ('Hashem'), soybean ('033'), navy bean ('Dehghan') and corn ('Sc704') were obtained from the Seed and Plant Improvement Institute (Karaj) then all seeds were powdered for use in artificial diet.

**Rearing methods and experimental conditions:** The eggs were acquired from a laboratory culture kept on an artificial diet at the University of Tabriz, Iran. Five separate stock cultures were maintained for two generations on artificial diets based on the seeds of five host plants before being used in the experiments. The experiments were conducted in a growth chamber  $(25 \pm 1^{\circ}C, 65 \pm 5\% \text{ RH}, \text{ and a photoperiod of 16:8 (L:D) hours).}$ 

Artificial diets contained powdered seeds of five different host plants (250 g), wheat germ (30 g) as protein and carbohydrate sources, sorbic acid (1.1 g) as an antimicrobial agent, ascorbic acid (3.5 g) as a vitamin source, sunflower oil (5 ml) as a preservative, agar (14 g) as a moisturizer, methylp-hydroxyl benzoate (2.2 g) as an antimicrobial agent, formaldehyde 37% (2.5 g) as an antimicrobial agent and distilled water (650 ml) (Teakle, 1991). Prepared artificial diets were kept refrigerated for no longer than two weeks before use.

Newly emerged larvae from the eggs laid by females, which had already been reared on five host plants were divided into five replicates (10 larvae per replicate). They were transferred into plastic containers (diameter: 15 cm, depth: 7 cm) which had a hole covered by a fine mesh net for ventilation. The third to sixth instar larvae were used to determine the nutritional indices of *H. armigera* on each host plant. The daily weight of larval foods and feces remaining at the end of each day were measured, and the amount of fresh food provided for each larva was also measured daily. To prevent cannibalism, the fourth to sixth instar larvae were kept individually in plastic tubes (diameter: 3 cm, depth: 5 cm).

The observations were recorded daily until the larval feeding finished and reached to the pre-pupal stage. All indices were calculated using dry weights. To estimate dry weight, additional specimens of each larval instar (third to sixth instars), feces produced and foods remaining at the end of each day were collected and weighed daily, oven-dried (48 hours at 60°C) and then reweighed. The nutritional indices were calculated as the following formulae (Waldbauer, 1968):

Relative growth rate (RGR) =P/  $(I_w \times T)$ 

Relative consumption rate (RCR) =  $E/(P \times T)$ 

Efficiency of conversion of ingested food (ECI) =  $(P/E) \times 100$ 

Efficiency of conversion of digested food (ECD) =  $[P/(E-F)] \times 100$ 

Approximate digestibility  $(AD) = [(E-F)/E] \times 100$ 

P= dry weight gain of larvae (mg), E= dry weight of food ingested (mg), F = dry weight of feces produced (mg),  $I_w$ = initial dry weight of larvae (mg), T= feeding period (day).

Statistical Analysis: Normality of the data was tested by Kolmogorov-Smirnov method. Difference among means was tested by one-way ANOVA (PROC GLM, SAS Institute). If significance difference was detected, multiple comparisons were made using the Duncan procedure ( $\alpha$ =0.05).

Nutritional indices of whole larval instars of *H. armigera* reared on five different host plants were used for cluster analysis. To classify the host plants, a factor analysis was carried out based on Ward's method. The cluster and factor analysis were done using SPSS16.0

#### **Result and Discussion**

Different host plants had significant effects on nutritional indices of *H. armigera* (Tables 1-5). The third instar larvae (Table 1) had the highest ECI ( $6.8\pm0.004$ ) (*F*=9.81; *df*=4,10; *P*<0.001) on cowpea and the highest ECD ( $9.9\pm0.012$ ) (*F* =3.88; *df*=4,10; *P*<0.037) on chickpea.

However, the lowest value of these variables in this instar was on corn (2.1±0.006 and 3.2±0.009, respectively). The larvae that fed on chickpea, had the lowest value of AD (17.2±0.10) (F=8.37; df=4,10; P<0.003). The results indicated that RGR value of the third instar larvae was not significantly different on five host plants tested. The highest value of food consumption was observed on cowpea and highest feces produced were on soybean.

The data presented in Table 2 showed that there were significant differences among nutritional indices of the fourth instar larvae of *H. armigera* on five host plants. The lowest ECI ( $2.4\pm0.002$ )(F=21.11; df=4,143; P<0.0001) and ECD ( $6.9\pm0.005$ ) (F=11.03; df=4,72; P<0.0001) was observed on corn and the highest ECD was on cowpea ( $46.4\pm0.10$ ). The larvae that fed on cowpea, had the highest RGR ( $0.836\pm0.062$ ) and the larvae reared on corn had the lowest RGR ( $0.532\pm0.044$ ) (F=5.24; df=4,118; P<0.0006). The highest AD was on corn ( $56.6\pm0.031$ ) and lowest on chickpea ( $34.6\pm0.043$ ) (F=3.99; df=4,121; P<0.004) (Table 2). The highest weight gain was obtained on cowpea. The larvae reared on soybean showed the highest value of food consumption and feces produced.

In the fifth instar larvae (Table 3), the highest RGR (0.778±0.044) (F=2.65; df=4,103; P<0.037) was on cowpea. The larvae reared on corn showed the lowest ECI (9.6±0.005) (F=51.58; df=4,62; P<0.0001) and ECD (14.5±0.001) (F=27.45; df=4,77; P<0.0001). The highest food consumption rate was on soybean and the lowest feces produced was on corn.

The results of nutritional indices of the sixth instar larvae (Table 4) showed that the highest RGR ( $0.563\pm0.052$ ) was on cowpea and the lowest was on corn ( $0.377\pm0.023$ ) (*F*=4.11; *df*=4,52; *P*<0.005). However, the AD value was the highest on navy bean (75.3±0.011) (*F*=7.94; *df*=4,18; *P*<0.0007). The highest ECI and ECD values of *H. armigera* were on cowpea ( $26.7\pm0.017$  and  $40.5\pm0.011$ , respectively) and the lowest ones were on corn ( $9.7\pm0.004$  and  $19.5\pm0.011$ , respectively) (*F*=54.81; *df*=4,60; *P*<0.0001 and *F*=17.72; *df*=4,33; *P*<0.0001). The least amount of feces was produced on chickpea.

Host plants	RCR	RGR	ECI%	ECD%	AD%	Р	Е	F
Cowpea	3.21±0.512a	0.243±0.007a	6.8±0.004a	8.8±0.005a	20.5±0.001b	0.608±0.0002a	15.57±0.002a	8.52±0.006a
Soybean	3.44±0.278a	0.209±0.082a	3.6±0.011b	7.7±0.025a	47.5±0.004a	0.631±0.0002a	12.74±0.006a	7.68±0.003a
Corn	6.90±0.195a	0.181±0.003a	2.1±0.006b	3.2±0.009b	64.5±0.045a	0.392±0.0009a	19.75±0.002a	7.09±0.001a
Navy bean	7.92±1.098a	0.191±0.003a	2.5±0.004b	5.6±0.001ab	57.6±0.12a	0.592±0.0001a	16.39±0.002a	8.42±0.001a
Chickpea	3.56±0.739a	0.216±0.006a	5.7±0.001a	9.9±0.012a	17.2±0.10b	0.473±0.0003a	17.67±0.002a	7.22±0.001a

Table 1. Nutritional indices (Mean ± SE) of the third instar larvae of Helicoverpa armigera on five different host plants

The means followed by the same letters in each column are not significantly different (P < 0.05, Duncan)

RCR= relative consumption rate, RGR= relative growth rate, ECI = efficiency of conversion of ingested food, ECD = efficiency of conversion of digested food, AD = approximate digestibility, P= dry weight gain, E= dry weight of food ingested, F= the dry weight of feces produced

Host plants	RCR	RGR	ECI%	ECD%	AD%	Р	Е	F
Cowpea	4.23±0.22b	0.836±0.062a	7.2±0.004b	46.4±0.10a	34.6±0.043b	4.37±0.0002a	40.65±0.002b	56.07±0.003b
Soybean	4.34±0.23b	0.670±0.031abc	7.7±0.005ab	22±0.022b	41.9±0.042b	4.01±0.0004a	39.85±0.002b	49.26±0.004b
Corn	13.01±0.86a	0.532±0.044c	2.4±0.002d	6.9±0.005c	56.6±0.031a	1.57±0.0001b	30.57±0.001a	70.09±0.001b
Navy bean	10.65±0.58a	0.602±0.064bc	5.4±0.005c	14.4±0.024bc	43.5±0.039b	3.96±0.0004a	54.73±0.002a	72.27±0.003a
Chickpea	4.86±0.52b	0.772±0.095ab	8.9±0.008a	37.9±0.075a	39.4±0.046b	3.60±0.0004a	35.86±0.005a	70.59±0.003b

Table 2. Nutritional indices (Mean  $\pm$  SE) of the fourth instar larvae of *Helicoverpa armigera* on five different host plants

The means followed by the same letters in each column are not significantly different (P < 0.05, Duncan) RCR= relative consumption rate, RGR= relative growth rate, ECI = efficiency of conversion of ingested food, ECD = efficiency of conversion of digested food, AD = approximate digestibility, P= dry weight gain, E= dry weight of food ingested, F= the dry weight of feces produced.

Host plants	RCR	RGR	ECI%	ECD%	AD%	Р	Е	F
Cowpea	0.924±0.041c	0.778±0.044a	23.2±0.010a	84.5±0.10a	27.5±0.037c	42.18±0.0009a	181.92±0.004a	131.21±0.009a
Soybean	1.11±0.014bc	0.703±0.026ab	18.3±0.006b	30.5±0.028b	31.9±0.028c	35.56±0.0004ab	178.61±0.006a	128.54±0.014a
Corn	2.53±0.15a	0.581±0.051b	9.6±0.005d	14.9±0.006b	69.1±0.020a	15.46±0.0009d	130.69±0.002b	57.99±0.003b
Navy bean	1.30±0.054b	0.641±0.053ab	14.5±0.001c	20.2±0.021b	54.3±0.026b	33.38±0.0009b	183.41±0.011a	125.50±0.003a
Chickpea	1.06±0.046bc	0.759±0.040a	19.6±0.003b	74.1±0.036a	29.5±0.020c	26.04±0.021c	173.75±0.013a	78.92±0.003ab

Table 3. Nutritional indices (Mean ± SE) of the fifth instar larvae of *Helicoverpa armigera* on five different host plants

The means followed by the same letters in each column are not significantly different (P < 0.05, Duncan)

RCR= relative consumption rate, RGR= relative growth rate, ECI = efficiency of conversion of ingested food, ECD = efficiency of conversion of digested food, AD = approximate digestibility, P= dry weight gain, E= dry weight of food ingested, F= the dry weight of feces produced

Host plants	RCR	RGR	ECI%	ECD%	AD%	Р	E	F
Cowpea	0.829±0.081c	0.563±0.052a	26.7±0.017a	40.5±0.011a	63.8±0.014b	43.68±0.0008a	136.21±0.013b	55.94±0.006c
Soybean	0.904±0.032c	0.447±0.038b	23.8±0.012ab	38.7±0.019a	67.7±0.039b	41.49±0.0002a	166.63±0.005ab	50.95±0.003c
Corn	2.05±0.29a	0.377±0.023b	9.7±0.004c	19.5±0.011b	70±0.015ab	20.73±0.0005b	217.09±0.017b	100.80±0.005b
Navy bean	0.831±0.026c	0.383±0.022b	20.3±0.04b	34.2±0.032a	75.3±0.011a	40.17±0.0019a	175.86±0.002ab	61.20±0.001c
Chickpea	1.14±0.11b	0.474±0.034ab	24.5±0.021a	39.3±0.038a	64.3±0.018b	38.47±0.001a	220.02±0.015a	135.84±0.006a

Table 4. Nutritional indices (Mean  $\pm$  SE) of the sixth instar larvae of *Helicoverpa armigera* on five different host plants

The means followed by the same letters in each column are not significantly different (P < 0.05, Duncan)

RCR= relative consumption rate, RGR= relative growth rate, ECI = efficiency of conversion of ingested food, ECD = efficiency of conversion of digested food, AD = approximate digestibility, P= dry weight gain, E= dry weight of food ingested, F= the dry weight of feces produced

Host plants	RCR	RGR	ECI%	ECD%	AD%	Р	Е	F
Cowpea	0.471±0.010c	7.59±0.15a	20.1±0.002a	83.4±0.080a	28.9±0.024c	57.89±0.0005a	263.73±0.004c	195.46±0.007bc
Soybean	0.562±0.015b	6.42±0.32a	14.4±0.004c	34.5±0.022c	34.3±0.023bc	49.10±0.0011ab	271.50±0.003c	190.73±0.013bc
Corn	0.924±0.024a	5.57±0.14a	7.5±0.002e	14±0.006d	55±0.015a	30.52±0.0007c	409.27±0.011a	172.99±0.006c
Navy bean	0.408±0.025d	6.42±0.63a	12±0.012d	48.2±0.080bc	37.9±0.033b	48.02±0.0011ab	349.37±0.006b	208.67±0.008b
Chickpea	0.519±0.014bc	6.87±0.18a	16.9±0.004b	57.5±0.073b	33.8±0.033bc	44.67±0.004b	388.46±0.011a	296.51±0.014a

Table 5. Nutritional indices (Mean ± SE) of whole larval instars of *Helicoverpa armigera* on five different host plants

The means followed by the same letters in each column are not significantly different (P < 0.05, Duncan)

RCR= relative consumption rate, RGR= relative growth rate, ECI = efficiency of conversion of ingested food, ECD = efficiency of conversion of digested food, AD = approximate digestibility, P= dry weight gain, E= dry weight of food ingested, F= the dry weight of feces produced.

# Dendrogram using Ward Method



Fig. 1. Dendrogram of five host plants based on nutritional indices of Helicoverpa armigera

The highest food consumption rate was observed on navy bean. The nutritional indices of whole larval instars (third to sixth instars) of *H. armigera* on five different host plants were summarized in Table 5. The larvae fed on five host plants showed no significant difference in RGR value. The lowest value of RCR ( $0.408\pm0.025$ ) (*F*=121.87; *df*=4,126; *P*<0.0001) was on navy bean compared with the other host plants. The highest values of ECI and ECD were on cowpea ( $20.1\pm0.002$  and  $83.4\pm0.080$ , respectively) (*F*=40.03; *df*=4,130; *P*<0.0001), and lowest ones were on corn (7.5 $\pm$ 0.002 and 14 $\pm$ 0.006, respectively) (*F*=25.79; *df*=4,99; *P*<0.0001). The highest food consumption rate and the least amount of feces were produced on corn.

The dendrogram of nutritional indices of whole larval instars of *H. armigera* showed two distinct clusters labeled A (including subclusters A1 and A2) and B. The cluster A consisted of subclusters A1 (cowpea and chickpea) and A2 (navy bean and soybean) and the cluster B included corn. Our results suggested that the cluster A is susceptible and cluster B can be categorized as a relatively resistant.

Variation in the quantity and quality of food eaten by an insect can affect its growth, reproduction, diapauses and migration (Nation, 2000; Golizadeh *et al.*, 2009; Soufbaf *et al.*, 2012). The ability of an organism to convert nutrients ingested, especially protein, to energy will positively influence its growth and performance (Sogbesan and Ugwumba, 2008). Polyphagous insects have high nutritional requirements for the growth, tissue maintenance, reproduction and energy. These chemicals are taken during feeding and the others are synthesized by organisms. Secondary plant metabolites can affect the activities of digestive enzymes, development, survival rate and reproduction of pests (Bernys and Chapman, 1994).

Our data showed significant differences among nutritional indices especially ECI and ECD, when *H. armigera* fed on the artificial diets based on the seed of five different host plants, so host plants could change nutritional requirements of the larvae of *H. armigera*. The ECI and ECD values showed significant difference in third to sixth instars, therefore different host plants can significantly affect the nutritional indices of *H. armigera*. Changes in nutritional requirements during development are probably responsible for the significant differences obtained for nutritional indices. Any changes in ECI and ECD values indicate the proportion of digested and ingested food converted into energy, thus no decrease and/or increase in values of ECI and ECD show that consumed secondary allelochemicals do not have any chronic toxicity effect (Koul *et al.*, 2004).

In most cases, the highest ECI and ECD value of the larvae of *H. armigera* was on cowpea. The decrease in ECI of *H. armigera* larvae may be resulted from the efficiency reduction in converting ingested food into growth. The larvae fed on corn had the lowest value of ECD compared with other host plants, indicating that these larvae have less efficiency for the conversion of digested food to their biomass. It is well known that the degree of food utilization depends upon the digestibility of food and the efficiency, which digested food, is converted into biomass (Batista Pereira *et al.*, 2002). Plant can synthesize secondary substances which play a major role in plant defense against insect pests which some of these include digestive enzyme inhibitors (Chougule *et al.*, 2003). Also, the values of ECD and ECI depend on the activity of digestive

enzymes (Lazarevic *et al.*, 2004) probably the presence of enzyme inhibitors lead to slow down the activity of digestive enzymes and result the reduction of ECD and ECI.

In the present study, the lowest RGR was on corn, which may be due to decreased consumption and/or ECI. Another possible reason for this reduction could be due to extension of larval period when amount of ingested food, which must be allocated to maintenance metabolism, was increased. The larvae fed on corn showed the highest RCR, probably due to unsuitable nutrient content and secondary substances. The high AD in larvae reared on corn might be due to compensation of nutrient deficiency. Maximum RCR, RGR and food consumption were observed at the fourth and fifth instars. It is due to greater rate of ingestion and maximum food intake during the fourth and fifth instars. During development of an insect, its nutritional requirements change, reflecting changes in food consumption and feeding behavior (Barton Browne and Raubenheimer, 2003). In fact, nutritional requirements are positively correlated with biomass and the duration of development (Kumar et al., 2008).

Among five different host plants, the lowest AD for whole larval instars was on cowpea, which is nearly similar to that reported by Naseri *et al.* (2010) for whole larval instars (second to fifth instars) of *H. armigera* fed on soybean var. 'Sahar' (0.353). The results indicated that the highest RGR was on cowpea. This finding is higher than that reported by Srinivasan and Uthamasamy (2005) on tomato accessions var. 'LE2' (1.19), suggesting that these hosts may be more suitable host plants perhaps because of higher nutritional quality of the food in comparison with tomato. The RGR for sixth instar larvae reared on cowpea is close that reported by Soleimannejad *et al.* (2010) for *H. armigera* (from the fifth instars to the end of larval stage) on soybean var. 'Clark' (0.59 $\pm$ 0.01).

The cluster dendrogram revealed that grouping different host plants within each cluster might be due to a high correspondence of physiological traits of host plants, whereas the separate clusters might represent significant variability in host plant suitability between clusters. The host plants categorized in subcluster A1 were the most suitable and those in subcluster A2 were fairly susceptible for *H. armigera*, while the host plant in cluster B had the least suitability. Corn that grouped in cluster B was the most resistance host plant because of nutrient deficiency and the presence of some secondary compounds. However, cluster A included the susceptible host plants due to the higher nutritional quality (Fig.1)

Our results on consumption rate, growth rate and digestibility indicated that the corn was less nutritive and cowpea was more nutritive than the other hosts. It may be due to the presence of some allelochemicals in this host plant acting as antixeotic and antibiotic agent or absence of primary essential nutrients for growth and development of cotton bollworm, suggesting that corn was unsuitable and cowpea was suitable host plants for *H. armigera*. The information could be used to manage the pest population to below the economic injury level. Also, these results provide data for selecting suitable host plants for rapid development, maximum survival, or high fecundity in order to use these individuals for mass rearing of natural enemies.

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