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Acceptability and anti-feedant effect of the drumstick "*Moringa oleifera* leaves towards the cotton leaf worm "*Spodoptera littoralis*(Boisd) under laboratory conditions.

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ABSTRACT

This study was carried on the acceptability and anti-feedant effect of Moringa oleifera (Lamk.) leaves as host plant towards the cotton leaf worm Spodoptera littoralis (Boisd.). The experiments were done on neonate and fourth instar larvae. Choice and non choice test for the larvae using tested plant in comparison with the control leaves (Ricinus communis) were used to indicate their preference towards M. oleifera leaves as host plant. The different biological criteria of S. littoralis 1st and 4th instar larvae on the tested host plant in comparison with control plant were studied under laboratory conditions. The obtained results show highly significant anti-feedant effects of M. oleifera leaves towards both studied instars in comparison with castor oil leaves as a control. Also, the percentage mortality of the larvae was very high and those of the 1st instar larvae failed to complete one generation and all of them died during 2nd and 3rd instars. Again the survival of the fourth instar larvae was significantly decreased in comparison to the control larvae. Nutritional indices of S. littoralis 4th instar larvae on M. oleifera leaves compared with the control plant leaves were studied under a temperature of 25± 2°C, R.H. of 65±5 % and 16:8 L: D photo period. The efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were significantly decreased in case of larvae fed moringa in comparison with the control larvae. M. oleifera leaves hindered the larvae from consuming a considerable amount of food. Therefore, the tested plant leaves may have allelochemics which act as phagodetterent for food ingestability. Also, M. oleifera leaves decreased the weight gain significantly in the treated individuals in comparison with the control. The relative consumption index (CI) increased in case of treated leaves in comparison with the control ones. These data may be due to consuming protein and fat contents resulting from severe starvation. Thus M. oleifera was considered to be unsuitable host plant for S. littoralis.

Keywords: Spodoptera littoralis, Moringa oleifera, Acceptability, Anti-feedant, Nutritional indices

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8(4)



INTRODUCTION

During recent years, some plants have been receiving global attention. The Drumstick tree "*Moringa oleifera*" (Lamk.) belongs to Moringaceae family commonly called Miracle tree. It is an important vegetable crop and is a fast–growing, drought resistant tree, native to the southern foothills of the Himalyas in North western India. It is the most widely distributed species [1]. It is cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. The leaves are the most nutritious part of the plant being a significant source of vitamin B, C, provitamin A as beta-carotene, vitamin K, manganese and protein, among other essential nutrients [2, 3, 4]. The cotton leaf worm "*Spodoptera littoralis* (Boisd.) is considered as one of the most destructive agricultural lepidopterous pest due to the damages it causes and control difficulties [5]. It causes a variety of damage as a leaf feeder. Phytophagous insects depend on plants as food which must fulfill their nutritional requirements for normal growth and development to occur. The differences in the susceptibility of different plants to the cotton leaf worm suggest that the insect can establish itself better on some plants than on others [6]. The degree of establishment of the insect would be determined by its ability to oviposit, survive and produce eggs on different plants. However, there are some aspects that might interfere with this rule. Nutrient deficiency and change in food composition strongly influence performance parameters such as survival, growth and development in phytophagous insects [7-10].

Secondary plant chemicals, nitrogen, water content and physical characters of plant leaves are considered to be detrimental factors significantly affecting the quality of the plant which in turn suppress insect growth and survival [11, 12]. Gut physio chemistry which may impact digestion in herbivourous insects varies among lepidopteran larvae [13]. The majority of literatures on the nutritional values of this pest's food deal with the effect of plant chemicals as gossypol [14] or nutrient deficiency as ascorbic acid deprivation [15] or even starvation [16] on the physiology and normal growth of the larva.

M. oleifera plant is not recorded as a host plant for *S. littoralis* larvae. However, several insects, mites and nematodes are registered as pests infesting *M. oleifera* tree [17; 18]. Quantitative analysis of the consumption of host plants by *S. littoralis* is an important factor used in studying and verifying larval plant varieties [19]. The quality and quantity of food consumed by the pest can affect its entire biology, rate of growth, development, reproduction and history [20]. Also, the various rearing conditions affected the different nutritional values [21]. The differences in the susceptibility of different plants to *S. littoralis* suggest that the insect can establish itself better on some than on others [22]. Host plant resistance among crop plants is a major part of integrated pest management .It is relatively constant, cheap, non polluting and is compatible with other methods of pest control. Developing resistant cultivars to *S. littoralis* larvae would supply an effective complementary approach in IPM to reduce the extent of losses caused by this pest [23, 24].

The objectives of the present study focus on the acceptability and supporting growth of *M. oleifera* leaves to the cotton leaf worm" *S. littoralis* "in a choice and non- choice test using castor bean leaves as a control plant. The studies were extended to the ability of this very useful tree to act as a host plant for *S. littoralis* larvae. The different biological aspects of both 1^{st} and 4^{th} instar larvae feeding on the tested plant leaves compared with the control leaves (castor bean leaves) are studied. Also, the nutritional indices of the newly moulted 4^{th} instar larvae are taken in consideration.

MATERIAL AND METHODS

The plant materials

M. oleifera leaves were kindly obtained from Prof. Dr. Aboelfetoh M. Abdalla, Professor at Technology of Horticulture Crops Department, NRC, Cairo, Egypt. The leaves were separately collected, cleaned and used daily for the present experiments.

Cotton leaf worm colony

Laboratory colony of the cotton leaf worm *S. littoralis* larvae used in the present study were obtained from a permanent culture maintained on castor oil leaves in the laboratory for several generations away from any insecticidal contamination in Department of Pests and Plant Protection NRC in Egypt. The experimental larvae were reared in an incubator at $25\pm 2^{\circ}$ C. according to [25].

July – August 2017 RJPBCS 8(4) Page No. 1007



Acceptability preference

Choice test

Newly moulted 4th instar larvae were given a choice between two different foods *M. oleifera* leaves and *Ricinus communis* leaves. Acceptability test was conducted in an arena (Fig.1a) constructed from round glass Petri dish 20 cm. in diameter and 3 cm in height with wax bottom were used. It is divided into 2 equal partitions by using hard card board sheet put inside the arena with an opening in the middle to give free movement for the larvae to choose the proper host plant according to [26]. Equal quantities of Moringa as well as Castor oil leaves were put each in each side of the arena. A moist piece of filter paper was put on the bottom of each side to prevent drying of the leaves. To assess larval preference, 10 newly moulted 4th instar larvae were placed in the center of each arena and given the choice between the two different foods to investigate if they have naïve orientation and settlement preferences. The orientation preference of the larvae was recorded after 1, 2, 4, 24 and 72 hours in the arena [27]. If the larva was not on a food source on the different recording time, it was recorded as a non- choice and was not used in determining the orientation preference. The acceptability and settlement preference test was replicated five times. The data obtained determined the relative percentage repellency or feeding deterrence was calculated according to Nerio, et al., [28].

> % repellency according to [28] $PR = \frac{Nc - Nt}{Nc} \times 100$ Nc + Nt

Where Nc = the number of larvae on the control plant after the exposure interval Nt = the number of larvae on the studied plant after the exposure interval

Non choice test

Biological criteria of S. littoralis reared on M. oleifera leaves

To test the efficiency of *M. oleifera* leaves on the growth and development of *S. littoralis* larvae two experiments have been done, the first is on neonate larvae and the second one is on the 4th instar larvae. The larvae were taken from the laboratory colony and were reared on the tested plant (Moringa leaves) as well as on castor bean leaves for comparison. Experiments were done in plastic cups 100 ml. capacity, and 10 first instar larvae were introduced to each cup. Five replicates were done for each host plant. In case of the fourth instar larvae, the insects were bred singly. Each larva was in a single plastic cup with the tested plant. 30 newly moulted 4th instar larvae were used for each treatment. Each larva was weighed to obtain the initial weight before starting the experiments. All cups were kept at constant temperature of $25\pm2^{\circ}$ C. 65+10 % R.H and 14: 10 L:D photoperiod until larvae started to pupate. Larvae were freely fed on the two host plants which were replaced daily to avoid excessive water loss. The different biological variables i.e. larval duration, survival and pupal weight were assessed. In each choice and non choice test, the experimental larvae were starved for 2 hours before starting the experiments.

Nutritional indices of the studied pest reared on *M. oleifera* leaves

To determine the effect of *M. oleifera* leaves as a host plant for *S. littoralis* larvae, the ingestability, digestability and other nutritional indices were studied. Ten newly moulted 4th instar larvae were selected, separated from each host plant and weighed to obtain their initial weight, individually isolated in plastic cups and maintained in a climate chamber at $25\pm2^{\circ}$ C, 60 ± 10 % R.H. and a photoperiod of 14: 10(L: D). The food either moringa or castor bean leaves were weighed daily (0.3 gm) and offered to each single larva. Accordingly 10 replicates were conducted for each tested plant. The development of the larvae was checked daily. Faeces were carefully separated from uneaten host, weighed and dried to a constant weight. Also, the remaining food was removed and stored. After 7 days, the larvae were weighed, killed by freezing and subsequently dried in an oven to a constant weight. The remaining uneaten diets were also dried at $55 - 60^{\circ}$ C for 72 hours until reaching constant weight indicating complete dehydration. At the same time , fresh and dry weight of 10 larvae were recorded to obtain the correction factor for initial dry weight, which was calculated from the average dry weight divided by the average fresh weight. The value was multiplied by all initial fresh weights of the larvae used in the experiments. All weight values were converted to dry weight values. The nutritional

July – August

2017

RJPBCS

8(4) Page No. 1008



indices were calculated according to the formula of Waldbauer, [29] and [19]. All calculations were based on the dry weight of the different data in mg.

Approximate digestibility (AD) = % assimilation efficiency or coefficient of digestibility

= <u>Amount of food consumed during experimental time -faeces X 100</u> Amount of food consumed

Efficiency of conversion of digested food (ECD) % to body matter = dry weight gain in mg X 100 Amount of food consumed –faeces

Efficiency of conversion of consumed food (ECI) $\% = \frac{dry \text{ weight gain in mg}}{X 100}$ Amount of food consumed

> Relative growth rate (RGR) = weight gain TA (CI) = Consumption index = F TA

Where

F= Food ingested (dry weight of food eaten) T= feeding period in days (duration of experimental period) A= mean dry body weight of the larva during feeding period W= dry weight gain for insect ECD measures the efficiency with which assimilated food is converted into insect tissues. ECI Measures the overall ability of insect to convert consumed food to the body tissues of the insect

Data Analysis

The biological and nutritional indices of S. littoralis reared on M. oleifera leaves and castor bean leaves as control were analyzed with one-way ANOVA test. Significant differences between treatments were determined using Duncan's test (P < 0.05).

RESULTS AND DISCUSSION

Choice test

Acceptability and Anti-feedant Activity

The results obtained in (Table1) show that S. littoralis larvae are able to discriminate between plants which are acceptable or non acceptable as food. After the 1^{st} , 2^{nd} and 4^{th} hours, only 40 – 42 % of the larvae settled on *M. oleifera* leaves while 60-58 % were settled on castor bean leaves. After 24 hours, the majority of larvae settled on Ricinus communis leaves (80%) and only 20 % are directed and settled on M. oleifera leaves. After, 48 hours, 98% of the larvae preferred castor bean leaves and only 2% are directed and settled on M. oleifera leaves(Fig.1b). This is in agreement with Dimetry, 1972 [6] and Douan et al., [22] who found that the differences in the susceptibility of different plants to S. littoralis suggest that the insect can establish itself better on some plants than on the others. On readily acceptable plants growth of the larvae is equally good, while on the less acceptable plants, growth is very prolonged. The larvae nipped at these leaves before rejecting them. After 72 hours, all larvae settled and feed on castor oil leaves. This may be attributed to the presence of some allelochemical substances present in M. oleifera leaves. The present findings are in agreement with Kamel and El- Gengaihi [30] who stated that the presence of fatty acids and sterols in moringa oil play a significant effect as anti-feedant on the tested insects. From (Table1), the percentage repellency of the tested plant was found to be 97.9 % after 48 hours increased to 100% after 72 hours. Dimetry, [6] mentioned that the slight acceptance of some plant and the consequent poor growth of S. littoralis larvae on them may be attributed either to the feeding deterrents or to the poor nutritional quality of the plant. In agreement with the present findings, Abd El-Aziz and Ezz El-Din [31] found that maytenus senegalensis had a chronic effect on the rate of S. littoralis growth, act as antifeedant and disturbed the larval development.

July – August

2017

RJPBCS

8(4) **Page No. 1009**



Jermy [26] added that the sensitivity to deterrents is a more important factor in determining the host range of phytophagous insects.

Time/ hours	Total No. of larvae on Castor bean leaves	% orientation	Total No. of larvae on Moringa leaves	% Orientation
1	30	60	20	40
2	30	60	20	40
4	29	58	21	42
24	40	80	10	20
48	49	98	1	2
72	50	100	0	0



(a) The sugessted arena without the larvae



(b) Orientation of *Spodoptera littoralis* larvae towards the two tested plants.

Fig. (1): Arena used for acceptability test

Non choice test

Biological studies on neonate larvae fed on M. oleifera leaves

The data present in (Table2) show that when 1st instar larvae were given the chance to feed and grow on *M. oleifera* leaves, 52 % mortality were recorded between the tested larvae. The duration of the 1st stadium was significantly elongated to be 5.19±1.04 days compared with 3.0±0.0 days in case of those larvae fed on castor bean leaves as control. Also, when the living larvae reached the second instar, 83.3 % mortality occurred between the larvae on the tested plant leaves compared with 10 % mortality for the control larvae. The duration of this instar was highly significantly increased to be 6.5±3.49 days compared with 1.29±0.46 days in case of the control. In the still living 3rd larval instar, 100% mortality recorded between the larvae and the 3rd larval stadium lasted 8.0±2.83 days compared with 4.0 days for the control. Again, the living larvae on the control plant complete their development to reach the pupal stage (Table2).

 Table 2: Different biological aspects of neonate Spodoptera littoralis larvae fed on Moringa oleifera leaves under

 laboratory conditions (Non Choice test)

	Mean Larval Duration in Days (Mean±SD)				
Larval instars	Moringa (Range)	Mortality%	Castor bean (Range)	Mortality %	
L1	5.19±1.036 ^{NS} (4-7)	52	3±0 (3)	0	
L2	6.57±3.49* (4-14)	83.3	1.29±0.46 (1-2)	10	
L3	8±2.83* (3-10)	100	4±0.66 (3-5)	2.5	
L4	-	-	2.78±0.42 (2-3)	0	
L5	-	-	4.51±1.08M(3-6)	0	
L6	-	-	4.98±0.91 (4-6)	0	
Pupa	-	-	12.88±1.9 (12-14)	0	

* A mean with an asterisk is significantly different from that of the corresponding controls at 5% level of probability (p≤ 0.05). L, larval instars. -, absence of survivors.

8(4)



Cespedes *et al.*, [32] mentioned that the death of treated insects caused by plant extracts may be due to the inability of the moulting bodies to swallow sufficient volume of air to split the old cuticle and expand the new one during ecdyses or to a metamorphosis inhibiting effect of the plant extract, especially sterols which is possibly based on the disturbance of the hormonal regulation. All these factors may be causing a disturbance in hormonal balance which influencing on development of treated insects.

From the foregoing results, it could be concluded that Moringa leaves cannot support growth well for *S. littoralis* larvae. This is due to the fact that food consumption was reduced perhaps by reducing growth and this in turn influences the food uptake, inhibition of enzyme activities and interference with protein metabolism (Nathan and Kalaivani, [33].

Biological studies on 4th instar larvae fed on *M. oleifera* leaves

The data obtained in (Table3) show the different biological aspects of the 4th larval instar of *S*. *littoralis* fed on Moringa leaves compared with the control leaves. The duration of the different instars of the pest was significantly elongated in case of larvae fed on *M. oleifera* leaves in comparison with those larvae fed on the control leaves. The total larval duration increased significantly being 21.55 days on the tested plant compared with 10.79 days in case of the control i.e. they took twice or more the time of larvae fed on the control diet. When the larval duration extended, the mortality rate increased. Again, the mortality between the tested larvae increased in all instars in comparison with the control ones and only 9.1 % of the larvae succeeded to pupate in comparison with 91.7 % in the control larvae (Table3).

Table 3: Different biological aspects of Spodoptera littoralis 4th instar larvae fed Moringa oleifera leaves under laboratory conditions (Non Choice test)

Larval	Moringa		Castor Bean		F-ratio
Instars	Duration in (Days)M±SD (Range)	Mortality %	Duration in (Days)M±SD (Range)	Mortality %	
L4	5.76±2.1** (2-10)	36.4	3.5±0.72 (2-5)	0.0	24.687
L5	8.79±2.61* (5-15)	35.7	3.05±0.80 (2-4)	8.33	90.09
L6	7±2.12* (5-12)	77.8	4.24±0.99 (3-6)	0.0	24.11
Pupa	12.33±1.53 ^{NS} (12-14)	90.9	12.5±3 (10-15)	0.0	0.009

* A mean with an asterisk is significantly different from that of the corresponding controls at 5% level of probability ($p \le 0.05$). L, larval instars.

Also, the pupal weight was significantly decreased in comparison to the control (Fig.2). This delaying in development of the treated larvae led to significant underweight pupae 132.33± 36.35 mg. compared with 263.53 mg for the control (F-ratio 62.88). These data are in agreement with those reported by (Abd El- Aziz and Ezz El-Din, [31] who found that *Maytenus senegalensis* had a deleterious effect on *S. littoralis* growth. They added that this host plant act as antifeedant and disturbed the larval development. Also, Marei *et al.*, [34] concluded that Jojoba and Sesame oils caused pronounced prolongation in both larval and pupal durations and finally led to underweight pupae of *S. littoralis*.

Food consumption and nutritional indices of S. littoralis 4th larval instar fed M. oleifera leaves

The data obtained in (Table4) illustrate that *M. oleifera* leaves hindered the larvae from consuming a considerable amount of food. The larvae consumed 201.8 ± 62.91 mg of Moringa leaves. On the contrary, larvae fed castor bean leaves (control host), their food consumption significantly increased to 354.78 ± 75.78 mg comparing with the larvae fed on moringa leaves. Therefore, it could be concluded that moringa leaves have allelochemics which act as phagodeterrent for food ingestability. Again the titer of secondary plant

July – August 2017

7 F

RJPBCS 8(4)

Page No. 1011



substances in Moringa leaves may affect the digestion. Our criteria in this respect are based on the weight of food consumed during the experimental time. The data obtained in (Table4) show that the percentage of dry weight consumed per larva with respect to food consumed in the control was significantly affected and decreased to be 56.88 % in case of larvae fed on Moringa compared with those fed on the control leaves. The disturbance in ingestability and digestibility had an indirect effect on the assimilation of food. The data obtained in (Table 4) show that the body weight gain was affected significantly. Larvae fed on M. oleifera leaves gained 15.9±6.75 mg of their body weight with respect to larvae fed on castor bean leaves 66.99± 23.3 mg. The dry weight gain of larvae fed the tested leaves were significantly (P < 0.05) less than those fed castor bean leaves. Schoonhoven and Meerman, [35] attributed the retardation in the larval growth due to the fact that the insect in general spent a considerable amount of energy to detoxify the allelochemics present in the host or diet. Generally if an allelochemic reduces ingestion, assimilation or efficiency of conversion of assimilated materials, it has prevented essential nutrients from being available to the insect [36]. Isman, [37] stated that any substance which reduced food consumption by the larvae can be considered as antifeedant. On the other hand in the present findings AD = 71.75 ± 15.88 mg. in larvae fed on Moringa compared with those larvae fed on the control leaves (54± 7.31mg.). These results were in agreement with those declared by [38 and 39] when larvae of S. eridania, or S. littoralis were bred on natural food other than cotton leaves, higher values of ADs were obtained. This was attributed to the increase in AD under the stress of sever starvation, the fact that insect requires a lot of energy to deal with the unfavorable food.

Table 4: Effect of Moringa oleifera leaves on the food consumption and nutritional Indices of the cotton leafworm
Spodoptera littoralis larvae under laboratory conditions

Nutritional indices	Moringa leaves Mean±SD	Castor Bean leaves Mean±SD	F- value
Dry wt. of food consumed/larvae (mg)	201.8±62.91*	354.78±75.78	23.11
Dry wt. of feaces (mg)	53.06±23.48*	160.6±30.48	75.09
Dry wt. of feaces with respect to food consumed %	23.72±8.054*	46±7.36	37.88
Dry wt. gain (mg)	15.9±6.75*	66.99±23.3	44.37
AD %	71.75±15.88*	54±7.31	9.40
ECD %	9.07±2.81*	42.75±16.96	34.68
ECI %	7.05±2.40*	21.59±9.58	37.49
RGR	0.22±0.069 ^{NS}	0.26±0.007	3.73
CI	3.24±0.91*	1.64±0.95	12.45

* A mean with an asterisk is significantly different from that of the corresponding controls at 5% level of probability ($p \le 0.05$).

(AD): Approximate digestibility.

(ECD): Efficiency of conversion of digested food.

(ECI): Efficiency of conversion of consumed food.

(RGR): Relative growth rate.

(CI) : Consumption index.



Fig. (2): Spodoptera littoralis pupae resulted from larvae fed on the tested plant leaves

a- Moringa oleifera leaves.

b- Castor bean leaves.



The results of the nutritional indices of the 4th larval instar were significantly decreased in larvae fed on moringa where ECD = 9.07± 2.81 while those fed castor bean leaves were significantly increased to 42.75± 16.96. Again ECI = 7.05± 2.4 and 21.59± 9.58 for larvae fed on moringa and control leaves respectively. ECI is a general index of an insect's ability to benefit from food consumed for development and growth, and ECD is an index of the efficiency of conversion of digested food into growth [33]. The inhibition of the process of converting digested food caused a significant reduction in the overall efficiency of ingested food accordingly the assimilation of food in the body tissue (ECI) was significantly reduced for larvae fed moringa leaves in comparison to the control larvae. The data obtained in (Tables 3 & 4) show that the larval instars are prolonged and the growth rate of the insect is reduced. This delaying in development of the treated larvae led to a significant underweight pupae 132.33± 36.35 mg. of *S. littoralis* compared with 263.53 mg for the control. The present data are in agreement with Khedr et al., [40] who stated that all the tested cotton genotypes decreased the feeding behavior of S. littoralis larvae in terms of consumption percentages, consumption rate (CR), growth rates (GR), efficiency of conversion of ingested and digested food (ECI and ECD), approximate digestibility (AD), and feeding deterrence (FDI) compared to the control. Abdel- Rahman and Al-Mozini, [41] found severely reduce GR, CR, and ECI in S. littoralis larvae treated with three plant extracts. These authors reported that reduction of digestion resulted from covalent bands with food proteins or digestive enzymes. This affected the dry weight gain of the larvae. The consumption index of the food consumed (CI) increased in the treated larvae in comparison to the control.

The present findings are in consistent with those obtained by (Yazdani *et al.*, [42, 43] who found that monoterpenes inhibited the efficiency of conversion of assimilated food of *Glyphodes pyloalis* (Walker) larvae, resulting in a low efficiency of conversion of ingested food (ECI). This affected the dry weight gain of the larvae. From the foregoing results, it could be concluded that the inhibition of growth in larvae offered *M. oleifera* leaves may be attributed to the reduction in the efficiency of conversion of digested food and efficiency of conversion of ingested food. After 7 days, the body weight of the larvae fed tested plant leaves was significantly reduced. They gained only 36.82% as much weight with regard to the control larvae. The treated larvae gave pupae with significantly (P < 0.5) low body weight.

The present findings are in agreement with the data obtained by (Ruan and Wu, [44] who stated that different nutritive values of the host plants may influence the rate of development. Singh and Parihar, [45] ascertained that the availability of different host plants plays an essential role in causing population outbreaks for polyphagous insects.

Also, analysis of nutritional indices can lead to understanding of the behavioural and physiological basis of insect response to host plants [46]. The present findings ascertain that lower acceptance of *S. littoralis* on *M. oleifera* leaves may be due to the presence of some secondary chemical substances in this host plant or may be due to the absence of primary nutrients necessary for growth and development. The present findings are in accordance with Silva *et al.*, [47] who found that the growth weight gain and efficiency in the conversion of ingested food were influenced by the different host plants in *Spodoptera fragiperda*. This clarifies the importance of the presence of a number of deterrent allelochemicals that are hindering insect development. Some important investigations are needed in the future to show the important elements present in Moringa leaves responsible for the deterrent or antifeedant effect towards *S. littoralis*.

CONCLUSION

The present work would lead to effective screening of plants having insecticidal properties, supports interest in the development of biopesticides from plants for protection of cultivated plants in Egypt and the active ingredients present in *M. oleifera* leaves are considered as pest alternatives for effective repellence and deterrence against insect pests. This will also contribute to enhance the economic value of the floral diversity.

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July – August 2017 RJPBCS 8(4) Page No. 1013



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8(4)

Page No. 1014