www.czasopisma.pan.pl PAN www.journals.pan.pl
POLSKA AKADEMIA NAUK

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 53, No. 2 (2013)
DOI: 10.2478/jppr-2013-0028

SUITABILITY OF *CENTELLA ASIATICA* (PEGAGA) AS A FOOD SOURCE FOR REARING *SPODOPTERA LITURA* (F.) (LEPIDOPTERA: NOCTUIDAE) UNDER LABORATORY CONDITIONS

Saeedeh Javar[1], Ahmad Said Sajap[1], Rozi Mohamed[1], Lau Wei Hong[2]*

[1] Faculty of Forestry, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia
[2] Faculty of Agriculture, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia

Received: October 31, 2012
Accepted: April 5, 2013

Abstract: When conducting entomological research, it is necessary to have a sufficient quantity of the desired pest. One of the pests commonly used in research is *Spodoptera litura*. Mass rearing of herbivorous caterpillars like *S. litura* requires large amounts of a suitable food source. Hence, the use of an easy-to-grow host plant would be most practical and reasonable. *Centella asiatica* known as 'pegaga' grows easily, produces plenty of leaves, and is available throughout the year in Malaysia. The suitability of *C. asiatica* as a food source for *S. litura* was evaluated by studying the biology of this pest on excised *C. asiatica* leaves, under laboratory conditions. *S. litura* completed its whole life cycle in 29 to 35 days. The mean pupal weight and the average number of eggs laid by a single female were 0.341 g and 1,930 eggs, respectively. Daily monitoring of the larval development stages as well as analysis on the width of head capsules, revealed the existence of six instars during the larval stages of *S. litura*. The cumulative survival rate for immature developmental stages was 80%, while the stage-specific survivorship was over 90%. This study demonstrated the use of 'pegaga' as a suitable new food source for when rearing *S. litura* larvae in the laboratory.

Key words: biology, *Centella asiatica*, rearing, *Spodoptera litura*, survival

INTRODUCTION

Conducting entomological experiments on pests means that there must be a sufficient quantity of the desired pests. To provide the high number of insects, an adequate amount of suitable food types is required. Mass rearing of insects is usually conducted on artificial or natural diets (Gupta *et al.* 2005). Most researchers prefer to rear the insect colonies on artificial diets since such diets are convenient, easy to prepare, and are available during all four seasons. However, artificial diets often contain various ingredients that are costly to obtain (Elvira *et al.* 2010). *Spodoptera litura* (Lepidoptera: Noctuidae) is among the most important pests with great economic impact, especially in the tropical regions. Continuous use of artificial diets has not only reduced the survival and the fecundity of *S. litura*, but has also increased the developmental period of the pest (Coudron *et al.* 2002; Gupta *et al.* 2005). The artificial diets used for rearing the insect colonies can also cause unnatural reactions. For instance, rearing *Spodoptera exigua* caterpillars on artificial diets resulted in a significant increase in the activity of their salivary enzymes, such as glucose oxidase, compared to plant-fed caterpillars (Merkx-Jacques and Bede 2005). For these reasons, the use of natural diets or host plants are useful alternatives for rearing the insect pests.

Castor bean (*Ricinus communis*) (Euphorbiaceae) is considered as a suitable host plant for *S. litura* larvae (Parasuraman and Jayaraj 1985; Zhou *et al.* 2010). The leaves of the castor bean have been widely used to rear the larvae of *S. litura*, under laboratory conditions (Rajagopal *et al.* 2002; Anand and Tiwary 2009). Even though castor is a suitable host for *S. litura* and can be grown on practically any soil, the plant requires a great deal of open space and has a slow initial growth (Council for Scientific and Industrial Research 1972; Phillips and Rix 1999). There is need to search for alternative host plants that can be grown easily. Our preliminary tests showed that *S. litura* has a high appetite for *Centella asiatica* (Umbelliferae), which is popularly known as 'pegaga' in Malaysia. The 'pegaga' is a small creeping herb with long stalks and orbicular-renniform green leaves. 'Pegaga' grows extensively in shady, marshy, wet places such as paddy fields (Singh *et al.* 2010). This perennial creeping herb is easily propagated through mature vegetative materials such as rhizomes, stem cuttings, and leaves (Zainal Abidin and Kamaruddin 2005). The 'pegaga' grows easily, produces plenty of leaves, and is available throughout the year in Malaysia. Large quantities of leaves can be readily made available for continuous rearing of the larval instars. There are no published reports on the suitability of 'pegaga' leaves as a food source for rearing the insects. Hence, this study was designed to determine the suitability of 'pegaga' for rearing *S. litura* by evaluating the effect of 'pegaga' on the developmental biology of the insect.

*Corresponding address:
ahsaid@putra.upm.edu.my

MATERIALS AND METHODS

Host plant

C. asiatica plants were obtained from the agriculture park (Taman Pertanian Universiti) of Universiti Putra Malaysia (TPU, UPM). Insecticides were not used on these plants during the entire study period. Excised leaves were washed with distilled water prior to use as food to rear the insect larvae.

Rearing of insects

A colony of *S. litura* was established from *S. litura* larvae that were obtained from the insect rearing center at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor. In the laboratory, the larvae were fed with excised 'pegaga' leaves that had been placed in plastic containers (12 cm diameter x 6.5 cm high). Fresh leaves were provided every 2 or 3 days. The mature larvae were transferred to another container containing vermiculate for pupation and adult emergence. The adult moths were kept inside containers (11 cm diameter x 10 cm high) that were covered internally by a piece of paper or tissue, for oviposition. The adult moths were fed with a 10% honey-water solution. Egg sheets were collected daily and placed in clean containers. The resulting neonates were allowed to complete their development on 'pegaga' leaves.

Development studies

The newly laid egg masses were placed inside plastic containers (12 cm diameter x 6.5 cm high) and were labeled with the date the eggs were laid. Eggs were incubated under laboratory conditions at 25±2°C and 65–70% relative humidity (RH). Upon hatching, the neonates were transferred individually to another container (7.5 cm diameter x 4 cm high) and were fed with freshly excised leaves. The larva inside each container was examined daily for ecdysis and upon molting, the larval length was measured. When the larvae turned into the pre-pupal stage, the container was filled with vermiculate. The pupation period was recorded. Upon emergence of the adults, pairs of female and male moths were placed inside a container (11 cm diameter x 10 cm high) for mating. The oviposition duration, the number of eggs laid, and moth longevity were recorded. A total of 50 larvae from two subsequent generations (25 larvae from each generation) were used. The survival of each individual larva was checked daily during the whole developmental-stage period. Percentage stage-specific survival was calculated by dividing the number of the individuals still alive at the end of each life stage by the number of specimens at the beginning of each life stage. The overall survival rate percentage was calculated by dividing the number of emerged adults by the number of eggs tested. The life cycle duration was obtained from 40 larvae which completed their development during the study.

Determination of larval instars

Once the larvae molted, the exuviae with head capsules were collected and kept in 70% ethanol. The width of the collected head capsules was measured using a Stereo Microscope with imaging software [NIS Elements BR 3.0 (Nikon)]. In addition, to distinguish different larval instars by length, the sizes of larvae were measured immediately after each ecdysis.

Statistical analyses

The data on developmental stages were statistically analyzed using IBM SPSS version 19.0 software. The data on larval head capsule widths were analyzed for variance based on the one-way ANOVA procedure, while the means were separated by Tukey's HSD test.

RESULTS

Life cycle duration

S. litura larvae fed on 'pegaga' completed their life cycle within 29 to 35 days with the average being 31 days (Table 1). The eggs hatched within 2–3 days and the larvae went through six instars with an average duration of 14.25 days. The pre-pupal stage lasted for an average of 3.37 days and the pupal stage lasted for 9.25 days. The pre-oviposition period and longevity of moths averaged 2.47 and 8.32 days, respectively.

Table 1. The life cycle duration of *S. litura* reared on 'pegaga' leaves

Stages	Development time [days]	
	mean ±SE	range
Egg incubation period	2.25±0.06	2.00–3.00
Larval instars	–	–
1st instar	2.45±0.07	2.00–3.00
2nd instar	2.32±0.08	2.00–4.00
3rd instar	2.57±0.07	2.00–3.00
4th instar	2.27±0.10	1.00–3.00
5th instar	2.22±0.10	1.00–4.00
6th instar	2.40±0.10	1.00–4.00
Total larval period	14.25±0.22	12.00–17.00
Pre-pupal period	3.37±0.09	2.00–4.00
Pupal period	9.52±0.16	8.00–11.00
Adult longevity	8.32±0.20	7.00–9.00
Pre-oviposition period	2.47±0.07	2.00–3.00
Total life cycle	31.87±0.25	29.00–35.00

SE – standard error

Pupal weight and adult fecundity

The mean pupal weight of *S. litura* larvae fed on 'pegaga' was estimated at 0.341±0.04 g, with a minimum and maximum of 0.30 and 0.39 g, respectively. The emerged moths started laying eggs within two days after female and male pairs were placed in the cage. Egg laying continued for 6 days. The daily oviposition pattern by a female *S. litura* moth is illustrated in figure 1. The peak oviposition time occurred between the third and sixth day after emergence. The total number of eggs laid by a female ranged from 1,717 to 2,190 eggs, with the average being 1,930.40 eggs.

Survival rate of immature stages

The overall survivorship data of *S. litura* showed that 80% of the laid eggs reached the adult stage (Fig. 2a). The stage-specific survival rates indicated survival rates of over 93% for all of the immature stages (Fig. 2b). Among the immature stages, the pre-pupal and pupal stages showed the lowest survival rate (93%). All other stages presented a 100% survival rate.

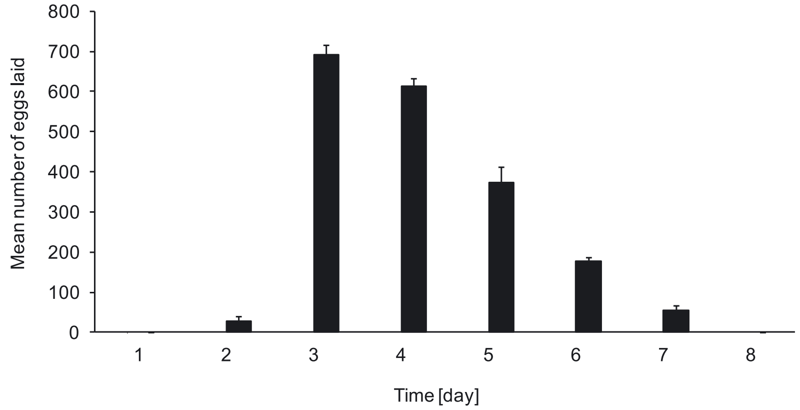

Fig. 1. Daily oviposition patterns of *S. litura*. Mean number (±SE) of eggs laid during one day intervals

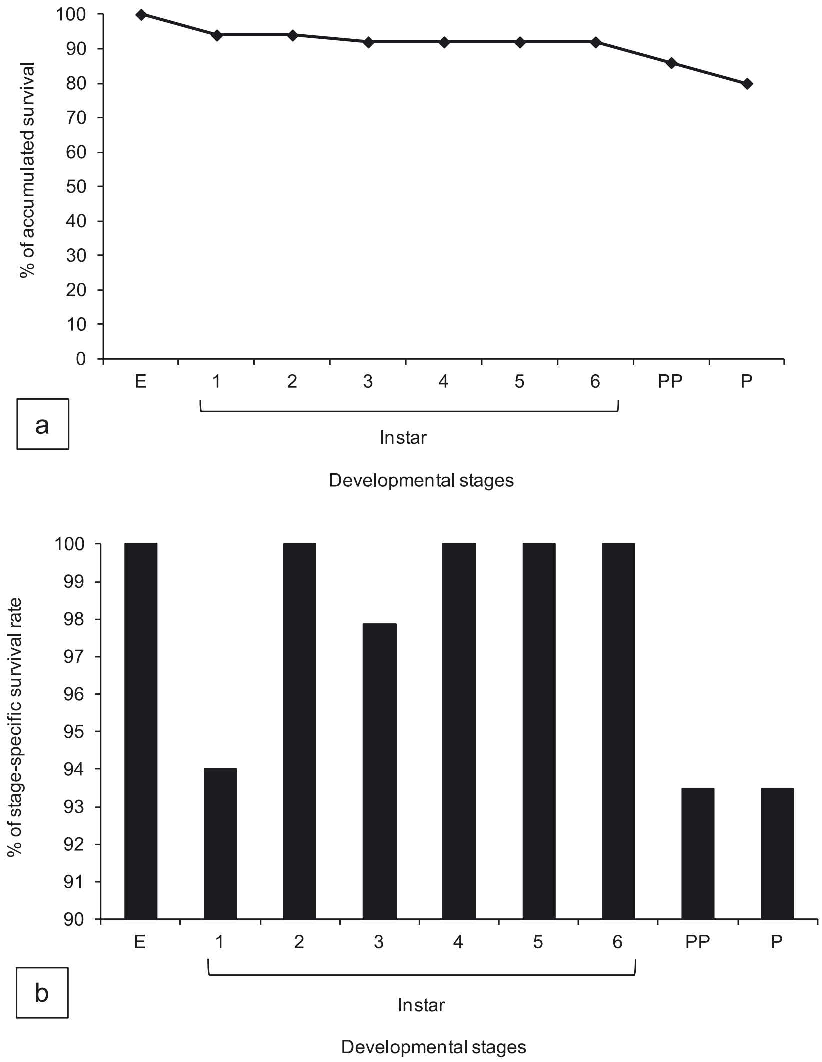

Fig. 2. Survival rates of immature developmental stages in *S. litura* when reared on 'pegaga' leaves: a) accumulated survivals, b) stage-specific survivals. Developmental stages: E – egg; 1–6 = 1–6 instars; PP – pre-pupa; P – pupa

Determination of larval instars

The frequency distribution of larval head capsule widths indicated the existence of six instars in *S. litura* reared on 'pegaga' leaves. No overlapping was observed in measurement extremes between instars. The mean head capsule widths computed for different instars (Table 2), showed significant differences between head capsule widths of different instars in *S. litura* ($F_{5/234}$ = 7,268.210; $p < 0.05$). In addition, comparison of the means using Tukey's test, revealed that the lowest head capsule size belonged to the first instar and the highest head capsule size belonged to the sixth instar, which indicated that the head capsule width increased along with the larval growth. The Dyar's growth ratios (Dyar 1890) were computed for head-capsule widths (Table 2). According to this ratio, head capsule width increases in a regular linear progression by a ratio of 1.5 (range 1.3 to 1.7) in successive instars. Dyar's ratios for the first to the sixth larval instar intervals in *S. litura* were 1.6, 1.6, 1.6, 1.5, and 1.5, respectively. The obtained Dyar's ratios indicated that *S. litura* larval growth rate followed Dyar's rule, and confirmed that none of the larval instars were disregarded. The average lengths of *S. litura* larvae immediately after ecdysis are presented in table 2. The average lengths of larvae varied from 3 mm at the time of the first ecdysis to 31.27 mm at the time of the last ecdysis.

Table 2. Body length of each instar immediately after ecdysis, head capsule width, and Dyar's ratio in *S. litura*

Larval instars	Larval length		Head capsule width [mm]		Dyar's ratio
	mean ±SE	range	mean ±SE	range	
I	3.00±0.01 a	2.80–3.20	0.25±0.00 a	0.24–0.27	
					1.60
II	5.15±0.04 b	4.50–5.50	0.40±0.00 b	0.36–0.40	
					1.67
III	7.72±0.14 c	6.00–9.50	0.67±0.00 c	0.63–0.74	
					1.69
IV	12.75±0.22 d	10.00–16.00	1.14±0.00 d	1.10–1.20	
					1.57
V	22.52±0.15 e	21.00–25.00	1.79±0.01 e	1.67–1.94	
					1.52
VI	31.27±0.15 f	28.00–33.00	2.73±0.024 f	2.30–3.00	

Means with different letters were significantly different ($p < 0.05$)

DISCUSSION

To understand the suitability of 'pegaga' as a food source for rearing *S. litura* larvae, results from this study were compared with previous results on established host plants. The data on the development of *S. litura* given pegaga as its food source, generally corroborated with that of results from previous studies (Table 3). The developmental period from egg to adult emergence ranged from 25.05 on castor to 32.08 days on groundnut (Garad *et al.* 1985), while Seth and Sharma (2001) recorded 26.1 days on castor. On 'pegaga', the duration between eggs until adult emergence was 29 days. Even though the developmental period from egg to adult emergence was about 3 to 4 days longer than those on castors, it was still shorter compared with those on groundnut. The larval period of larvae that were reared on 'pegaga' was also within the range of that larval period recorded from larvae fed on its common host plants. The larval period varied from 10.98 on castor (Patel *et al*. 1973) to 30 days on tobacco (Rattan and Nayak 1963). On pegaga, the larva passed through six instars and completed all the stadia within 14.25 days. This was only a day longer than those reared on artificial diets (Shorey and Hale 1965; Etman and Hopper 1979). In fact, it was shorter than those reared on common host plants such as tobacco, cowpea, and sweet potato (Table 3). Small variations in the pupal period were observed when results from this study were compared with the results from previous studies. Apart from those reared on perilla, with a recorded pupal period of 13.8 days (Bae and Park 1999), other host plants including 'pegaga' and artificial diets supported the pupal development that lasted less than 10 days. Hence, the overall development of *S. litura* on 'pegaga', including its larval and pupal periods, clearly indicates that 'pegaga' is a suitable host plant for rearing the insect. In addition to its comparable developmental period, *S. litura* reared on 'pegaga' had a pupal weight that was comparable to the pupal weight of those reared on castor and okra (Garad *et al.* 1985). The pupal weight was within the range of 0.32 to 0.37 g. The fecundity was lower than those reared on the artificial diet of Chu and Yang (1991) but higher than those reared on an artificial diet of Bae and Park (1999). Except for castor, 'pegaga' is a better host plant for egg production than sunflower (684 eggs) and groundnut (878 eggs) (Seth and Sharma 2001). *S. litura* also recorded a high immature survival rate of more than 93% and fecundity of 1,930 eggs per female when they were reared on 'pegaga'. The average number of eggs laid per female has been reported to be 1,038 (Sankarperumal *et al.* 1989), 2,088 (Seth and Sharma 2001), and 3,166.8 (Garad *et al.* 1985) eggs per female when *S. litura* larvae was fed on castor. While those which were fed artificial diets laid from 803 eggs (Bae and Park 1999) to 5995 eggs (Chu and Yang 1991). The overall performance of *S. litura* fed on 'pegaga' indicated that 'pegaga' is a suitable host plant for rearing the insect pest. It is probable that the nutrient content of 'pegaga' is comparable to that of its common host plants. Small variations among the results are expected. Xue *et al.* (2010) suggested that the developmental period of an insect, even within the same insect species, feeding on the same host, not only can be affected by the different environmental conditions, such as temperature, but also the strains or biotypes of the insect.

Table 3. The duration of egg to adult emergence, the larval, and the pupal periods of *S. litura* when the larvae fed on 'pegaga' and on previously established host plants

Developmental stage	Host plant	Temperature [°C]	Time [day]	References
Egg to adult emergence	pegaga	25±2	29	*
	castor	26.8±2	25.07	Garad *et al.* 1985
	sunflower		26.66	
	okra		30.03	
	groundnut		32.80	
	castor	26.8±1	27.2	Seth and Sharma 2001
Larval period	pegaga	25±2	14.25	*
	cabbage	26	13.3	Xue *et al.* 2010
	cowpea		15.8	
	sweet potato		17.5	
	tobacco		23.2	
	tobacco	26	19.3	Chen *et al.* 2002
	tobacco	23	30	Rattan and Nayak 1963
	cowpea	27	19.55	Shahout *et al.* 2011
	artificial diet	28	12.2	Etman and Hooper 1979
	artificial diet	–	13.4	Shorey and Hale 1965
	castor	26.8±2	11.50	Garad *et al.* 1985
	castor	28.6	10.98	Patel *et al.* 1973
	castor	26.8±1	15.8	Seth and Sharma 2001
	castor	15–26	23.9	Cardona *et al.* 2007
Pupal period	pegaga	25±2	9.60	*
	artificial diet	–	9.7	Shorey and Hale 1965
	artificial diet	28	7.7	Etman and Hooper 1979
	castor	26.8±2	9.24	Garad *et al.* 1985
	groundnut		8.98	
	okra		9.5	
	sunflower		9.5	
	perilla	24	13.8	Bae and Park 1999
	soybean	32	7.4	
	castor	26.8±1	7.9	Seth and Sharma 2001

*the result was obtained in this study

CONCLUSION

The biological parameters of *S. litura* including life cycle duration, pupal weight, female fecundity, survival rates, and the number of larval instars were studied towards assessing the suitability of 'pegaga' for the larvae of *S. litura*. The results were compared with similar parameters reported from other host plants especially castor, a plant that has been considered as the most suitable host for *S. litura*. Favorable results were obtained for all biological parameters of *S. litura* when the larvae were fed on 'pegaga'. Moreover, the insect pest went through a normal life cycle without impaired morphological or reproductive attributes during the process of mass rearing. Therefore, based on the results and easily available 'pegaga' leaves compared to castor leaves, 'pegaga' is recommended as a suitable alternative host plant to maintain *S. litura* colonies under laboratory conditions.

ACKNOWLEDGEMENTS

We wish to thank the staff of the insect rearing center at the Malaysian Agricultural Research and Development Research Institute (MARDI) for providing the insects, and the staff of the Agriculture Park, Universiti Putra Malaysia, for supplying the 'pegaga' leaves.

REFERENCES

Anand R., Tiwary B.N. 2009. Pathogenicity of entomopathogenic fungi to eggs and larvae of *Spodoptera litura*, the common cutworm. Biocontrol Sci. Technol. 19 (9): 919–929.

Bae S.D., Park K.B. 1999. Effects of temperature and food source on pupal development, adult longevity and oviposition of the tobacco cutworm, *Spodoptera litura* Fabricius. Korean J. Appl. Entomol. 38: 23–28.

Cardona E.V., Ligat C.S., Subang M.P. 2007. Life history of common cutworm, *Spodoptera Litura* Fabricius (Noctuidae: Lepidoptera) in Benguet. BSU Res. J. 56: 73–84.

Chen Q.J., Yang J.Q., Zhang J.Z., Zhang Y.Z., Chen J.H. 2002. Effect of temperature on laboratory population of *Spodoptera litura* (Fabricius) in tobacco fields. Tob. Sci. Technol. 2: 42–45.

Chu Y.I., Yang S.C.O. 1991. Ovipositional biology of the tobacco cutworm (*Spodoptera litura* (F.)). Chin. J. Entomol. 11 (3): 188–196.

Coudron T.A., Wittmeyer J., Kim Y. 2002. Life history and cost analysis for continuous rearing of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) on a zoophytophagous artificial diet. J. Econ. Entomol. 95 (6): 1159–1168.

Council for Scientific and Industrial Research. 1972. The Wealth of India. A Dictionary of Indian Raw Materials & Industrial Products. Vol. 9. Publications & Information Directorate, Council for Scientific and Industrial Research, New Delhi, India, 472 pp.

Dyar H.G. 1890. The number of moults of lepidopterous larvae. Psyche 5: 420–422.

Elvira S., Gorria N., Munoz D., Williams T., Caballero P. 2010. A simplified low-cost diet for rearing *Spodoptera exigua* (Lepidoptera: Noctuidae) and its effect on *S. exigua* nucleopolyhedrovirus production. J. Econ. Entomol. 103 (1): 17–24.

Etman A.A.M., Hooper G.H.S. 1979. Developmental and reproductive biology of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Aust. J. Entomol. 18 (4): 363–372.

Garad G.P., Shivpuje P.R., Bilapate G.G. 1985. Larval and post-larval development of *Spodoptera litura* (Fabricius) on some host plants. Proc. Indian Acad. Sci. 94 (1): 49–56.

Gupta G.P., Rani S., Birah A., Raghuraman M. 2005. Improved artificial diet for mass rearing of the tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae). Int. J. Trop. Insect Sci. 25 (1): 55–58.

Merkx-Jacques M., Bede J.C. 2005. Influence of diet on the larval beet armyworm, *Spodoptera exigua*, glucose oxidase activity. J. Insect Sci. 5 (48): 1–9.

Parasuraman S., Jayaraj S. 1985. Effect of host plants on the biology of *Spodoptera litura* Fabricius. Cotton Dev. 14 (4): 37–40.

Patel R.C., Patel J.C., Patel J.K. 1973. Biology and mass breeding of the tobacco caterpillar, *Spodoptera litura* (F.). Israel J. Entomol. 17: 131–142.

Phillips R., Rix M. 1999. Annuals and Biennials. Macmillan, London, England, 288 pp.

Rattan L.A.L., Nayak G.N. 1963. Effect of host plants on the development of caterpillars of *Prodenia litura* F. and their susceptibility to different insecticides. Indian J. Entomol. 25: 299–306.

Rajagopal R., Sivakumar S., Agrawal N., Malhotra P., Bhatnagar R.K. 2002. Silencing of midgut aminopeptidase N of *Spodoptera litura* by double-stranded RNA establishes its role as *Bacillus thuringiensis* toxin receptor. J. Biol. Chem. 277 (49): 46849–46851.

Sankarperumal G., Baskaran S., Mohandoss A. 1989. Influence of host plants on the organic constituents and fecundity of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). p. 393–396. In: Proc. Indian Natl. Sci. Acad. B 55 (6): 393–396.

Seth R.K., Sharma V.P. 2001. Inherited sterility by substerilizing radiation in *Spodoptera litura* (Lepidoptera: Noctuidae): bioefficacy and potential for pest suppression. Fla. Entomol. 84 (2): 183–193.

Shahout H.A., Xu J.X., Yao X.M., Jia Q.D. 2011. Influence and mechanism of different host plants on the growth, development and, fecundity of reproductive system of common cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Asian J. Agric. Sci. 3 (4): 291–300.

Shorey H.H., Hale R.L. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. J. Econ. Entomol. 58 (3): 522–524.

Singh S., Gautam A., Sharma A., Batra A. 2010. *Centella asiatica* (L.): a plant with immense medicinal potential but threatened. Int. J. Pharm. Sci. Rev. Res. 4 (2): 9–17.

Xue M., Pang Y.H., Wang H.T., Li Q.L., Liu T.X. 2010. Effects of four host plants on biology and food utilization of the cutworm, *Spodoptera litura*. J. Insect Sci. 10 (22): 1–14.

Zainal Abidin H., Kamaruddin H. 2005. Pegaga (*Centella asiatica*). p. 70–76. In: "Penanaman Tumbuhan Ubatan dan Beraroma" (Y. Musa, M. Muhammad Ghawas, P. Mansor, eds.). MARDI, Malaysia, 149 pp.

Zhou Z., Chen Z., Xu Z. 2010. Potential of trap crops for integrated management of the tropical armyworm, *Spodoptera litura* in tobacco. J. Insect Sci. 10: 1–10. DOI: 1673/031.010.11701.