

Journal of Biological Control, 38(4): 462-469, 2024, DOI: 10.18311/jbc/2024/44991

Research Article

Evaluation of different diets for dsRNA-based gene silencing assays in brinjal shoot and fruit borer, *Leucinodes orbonalis*

N. VEERAMANIKANTA REDDY[1,2], T. C. SUMAN[2,3], R. GANDHI GRACY[2], Y. K. YADU[1] , T. VENKATESAN[2*] and S. N. SUSHIL[2]

[1]*Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Purena – 492012, Chhattisgarh, India*

[2]*Division of Genomic Resources, ICAR- National Bureau of Agricultural Insect Resources, Bengaluru – 560024, Karnataka, India*

[3]*Department of Plant Biotechnology, University of Agricultural Sciences, Bengaluru – 560065, Karnataka, India*

**Corresponding author E-mail:* tvenkat12@gmail.com

ABSTRACT: The shoot and fruit borer of eggplant, *Leucinodes orbonalis,* is known to cause huge crop losses and reduce the marketability of the produce. In the current scenario of increased insecticide resistance in pests and concern for ecological sustainability, pest management using target-specific biomolecules such as double-stranded RNA (dsRNA) can be an efficient strategy. To conduct the preliminary lab studies using dsRNA, a diet used for bioassays is crucial as it should support insect growth and development while efficiently delivering the test material. The present study evaluated Artificial Diet (AD) and Potato Diet (PD) through a life table study. The stability of the dsRNA in the diets was assessed at 6, 12 and 24 h post mixing with the diets. The insect survival (62.6%), fecundity (65.48±3.15), sex ratio (1.3:1), and developmental parameters like net reproductive rate were found to be better for PD-reared individuals in comparison to AD-reared insects. The dsRNA was found to be stable for 24 h in AD, while it degraded at 12 h post mixing in PD. Therefore, the AD diet can be used for dsRNA oral feeding and can be replaced with a new diet at every 24 h, and for further rearing after dsRNA feeding, the PD can be used.

KEYWORDS: Artificial diet, brinjal shoot and fruit borer, dsRNA, net reproductive rate, survival

(Article chronicle: Received: 16-07-2024; Revised: 15-10-2024; Accepted: 22-10-2024)

INTRODUCTION

Brinjal shoot and fruit borer, *Leucinodes orbonalis* (Lepidoptera; Crambidae) is a key pest of brinjal, distributed in Asia, Africa and European countries, causing crop yield losses to an extent of 93% (Mally *et al*., 2015; Kodandaram *et al*., 2017; Prodhan *et al*., 2018). Muhammad *et al*. (2020) reported that a total of 30-40 sprays of synthetic insecticides were done by farmers to manage this sturdy pest in Bangladesh. This indiscriminate and ceaseless insecticide application not only posed a threat to humans and the environment but also resulted in the development of insecticide resistance and worsened the fate of pest management in brinjal (Kariyanna *et al*., 2020). More sustainable and safer biomolecules that possess minimal or zero effect on non-target species can ensure environmentally responsible pest management. RNA interference, a post-transcriptional gene silencing mechanism can be a way forward to achieve the goal. The dsRNA-based silencing of major genes involved in growth and development can bring down the pest load without any appreciable risk to non-target natural fauna. The delivered dsRNA will be cleaved into 18-24 bp length siRNA which complementarily binds to the mRNA of the target gene and thus hinders the gene expression ultimately leading to the death of the target insect (Elbashir *et al.,* 2001; Willkomm *et al.,* 2016).

To achieve effective pest management, in addition to researching the pest down to the transcript level and verifying essential genes for growth, development as well as synthesizing dsRNA for the target gene, a well-designed dsRNA delivery methodology is essential. For the study to be successful, selecting a convenient and reliable method of dsRNA delivery is much needed. The method used to assess the efficiency of dsRNA, should not alter the pest biology, change the form of test material used and should also be feasible for handling. Natural diet brinjal is perishable, has variable (varieties) composition and may not be available around the year. Artificial diet solves these problems and can yield the same quality of insects around the year. dsRNA delivery through artificial diets was proven successful in

pests like *Diabrotica virgifera virgifera*, *Bemisia tabaci*, *Helicoverpa armigera*, *Spodoptera exigua* and *Bactrocera dorsalis* (Bolognesi *et al*., 2012; Christiaens *et al*., 2014; Jing & Han, 2014; Tian *et al*., 2009). Oral delivery of dsRNA is labour-saving, cost-effective and is also applicable for large-scale gene screening for pest management (Tian *et al*., 2009).

Leucinodes orbonalis is an internal borer. Assays conducted using natural diets like brinjal or potato hinder taking observations for daily mortality, and morphological variations in larvae etc., This purpose can be solved by conducting experiments using artificial diets. Preparing a suitable artificial diet for monophagous pests like *L. orbonalis* is difficult. After screening a few artificial diets available in the literature, we had a satisfactory result with the AVRDC artificial diet (Talekar *et al*., 1999) and the whole Potato Diet (PD). Further modifying the AVRDC diet, we were able to come up with a modified Artificial Diet (AD) which yielded good insect populations upon rearing under laboratory conditions. A comprehensive life table study, dsRNA stability in artificial diets and an assessment on how often diets need to be replaced to ensure proper and continuous dsRNA intake is lacking. In this study, we have investigated the potential of diets for laboratory rearing of *L. orbonalis* through life table analysis and also tested these diets for dsRNA-based bioassays. An age-stage, two-sex life table has advantages over the traditional ones, which are only based on females' life studies. (Chi, 2014). The important parameters of insect life history, such as intrinsic rate of increase (r), Net Reproductive Rate (R_0), and Gross Reproduction Rate (GRR) were estimated for both diets.

MATERIALS AND METHODS

Preparation and composition of modified artificial diet

The diet as defined by AVRDC has been modified (Talekar *et al*., 1999). We attempted to increase the amount of brinjal in the diet by reducing the amount of pulse flour because *L. orbonalis* is a pest of brinjal. Immature brinjals were chopped, sun-dried until crispy, and then processed into a fine powder. After being sieved, the powder was refrigerated until needed. The components were those indicated in Table 1.

Definite quantities of ingredients were weighed (Table 1) and added to a mixer jar. To this 150 mL of distilled water was added and blended into a paste. On the other side, 4g of agar was added to 100 mL of distilled and brought to a boil. This agar solution upon cooling to 60 ˚C was added to the blended paste and mixed thoroughly. To this, the antimicrobial components were added and dispensed 1mL into each well of the bioassay tray.

Table 1. Composition of artificial diet (AD)

S. No.	Particulars	Quantity
1	Brinjal powder	15.0 g
2	Chickpea flour	25.0 g
3	Yeast	2.5 g
4	Sorbic acid (HIMEDIA)	0.5 g
5	Ascorbic acid (SDFCL)	0.5 g
6	Methyl para benzoxy hydrate (HIMEDIA)	0.8 g
7	Vitamin E (AQUALAB)	1 capsule
8	Multivitamin (GSK Labs)	1 capsule
9	Agar (HIMEDIA)	4 g
	Antimicrobial	
10	Formaldehyde (4%) (SDFCL)	0.5 mL
11	Carbendazim	0.05 g
12	Tetracycline	0.01 g
13	Distilled water	250 mL

Biology and life table study of *L. orbonalis*

The newly hatched larvae were transferred onto 0.5 mm thick potato slices or artificial diet (AD) cake, up to 10 neonates per slice using a camel brush. These slices were placed on tissue paper in a container (5 x 10 cm) and maintained under controlled conditions at 27±1ºC and 70-80% relative humidity . Later 3rd instar larvae were collected from these slices and used for further experiments. To simulate the bioassay, potato diet (PD) (one 0.5 cm^3 piece per well) and AD (1 mL per well) were added to wells of bioassay trays. The freshly moulted third instar larvae were collected from the respective diets, starved for two hours and transferred into wells containing the test diets. A total of 75 larvae were used for each diet assay (Figure1).

The potato cubes were changed every alternate day and wells were cleaned to maintain aseptic conditions till the larva pupate. The artificial diet was changed every 4th day till pupation. The pupae were collected, sexed and kept in a container till adult emergence. The observations were taken for larval total developmental duration, sex ratio, pupal duration, adult longevity, fecundity and total lifespan.

The pupae were sexed based on the distance between the genital slit and the last abdominal segments where the gap was more for males and very less space for the female pupae. (Singla *et al*., 2018) (Figure 2). Three males and females were transferred to mating and oviposition chambers which were provided with 10 per cent honey solution and water on the cotton swabs. (Rahman *et al*., 2011). The chambers were covered with black cotton cloth which also served as

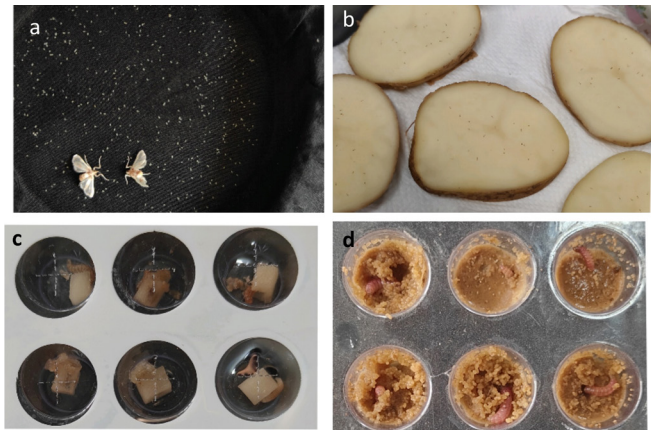


Figure 1. **(a)** Female adults and eggs laid on the cloth; **(b)** neonate rearing on potato pieces; **(c)** larvae rearing on potato cubes; **(d)** larvae rearing on a modified artificial diet.

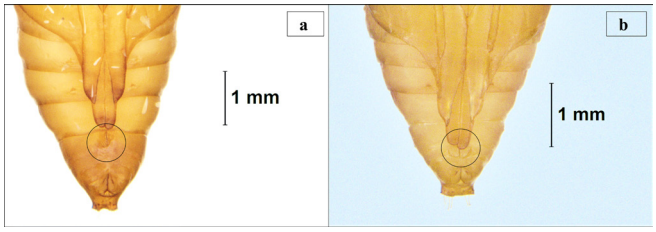


Figure 2. *L. orbonalis* pupal ventral view. **(a)** Female pupa; **(b)** Male pupa (encircled area shows the genital pore).

an oviposition site (Figure 1a). Every day the adults were transferred into new containers till they died. The eggs along with the containers were kept for hatching.

The life table data on egg hatching, larval and pupal development duration, survival, adult longevity, fecundity, etc., were recorded and analysed with age-stage and two-sex life table (Chi, 1988; Chi & Liu, 1985) using the TWOSEX-MS Chart version 2024/5 (Chi, 2014). A total of 75 individuals (N) were used in each treatment. The age-stage-specific survival rate (Sxj), life expectancy (Exj), age-stage reproductive value (Vxj), and other population parameters were calculated. This program applies basic statistics and the output for all the parameters (means and standard error) discussed in this paper was estimated at 10000 bootstraps. The developmental periods and reproductive parameters obtained from two diets were taken for age-stage and two-sex life table study. The two-sex MS chart software was used for this analysis.

RNA isolation and cDNA synthesis

The RNA was isolated using 1000 µl of RNAiso Plus (Takara, Japan). The purity and integrity were assessed using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies Inc., USA) and 1.2% (w/v) agarose gel electrophoresis, respectively. cDNA was synthesized by using 1µg of total RNA using a Prime Script™ RT reagent kit with a gDNA eraser (Takara) following the manufacturer's protocol.

dsRNA synthesis

The target gene *L. orbonalis E75* (411 bp) was PCR amplified using cDNA as a template with respective forward and reverse primers having T7 region on their 5'end with the cycling conditions: 3 minutes of initial denaturation at 95ºC, 34 cycles of 95ºC for 30 sec, 55ºC for 30 sec, and 72ºC for 1 min, followed by a final extension step at 72ºC for 10 min. The PCR product was purified using Nucleospin gel and a PCR cleanup kit. dsRNA was synthesized and purified using this PCR product as a template with MEGAscript® RNAi kit following the manufacturer protocol. The obtained dsRNA was quantified using NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies Inc., USA) and the integrity was checked using 1.2% (w/v) agarose gel electrophoresis and stored at -20ºC until further use.

Testing the stability of dsRNA on diets

The synthesized dsRNA was added 500 µl (100 ng/µl concentration) each to three separate 1.5 mL tubes. Later 0.5 g each of potato, brinjal and modified artificial diet was added to the tubes. The tube with only dsRNA was used as a control. These tubes were kept at room temperature and the quality of dsRNA from the respective tubes was assessed at 6, 12 and 24 hours by checking the stability on 1.2% agarose gel (Cao *et al*., 2018).

RESULTS AND DISCUSSION

Biology and life table parameters as studied in different diets

The *L. orbonalis* is majorly known to feed on eggplant fruit and also on related species such as other solanaceous crops including potato and tomato (Boopal *et al*., 2013; Rahman *et al*., 2011). Its biology includes an egg, 5 larval instars, a pupa and an adult with a life cycle span of 26 to 42 days depending on the seasons (Gupta & Kauntey, 2007; Mannan *et al*., 2015). A new and environmentally friendly management approach has a become need of the point. dsRNA-based gene silencing is a novel strategy in insect pest management. This technology is at a stage where rapid laboratory-level assays are being taken up at different centres in India and abroad. There is a prior necessity to devise potential bioassay methods to validate silencing of target genes. In this study, we have investigated the potential of a modified artificial diet and potato for lab rearing as well as to serve as a diet in dsRNA-based bioassays. As many studies indicate potatoes as suitable lab hosts, we have used PD instead of brinjal for the comparison with AD.

Table 2. Developmental time and fecundity of *L. orbonalis* reared on different diets

Particulars	Potato Diet (PD)	Modified Artificial Diet (AD)
Larval duration (days)	12.11±0.11	13.44±0.12
Pupal duration (days)	7.72±0.08	7.88±0.10
Pre-adult duration (days)	23.67±0.16	25.23±0.16
Total longevity male (days)	27.95±0.25	29.56±0.26
Total longevity female (days)	29.37±0.15	30.23±0.34
Sex ratio (female: male)	1.35:1	1.22:1
Female adult longevity (days)	5.7±0.13	5.0±0.26
Oviposition days	2.74±0.10	2.30±0.18
Fecundity (eggs/female)	65.48±3.15	52.36±5.93

Data given as **(mean ± S.E.);** S.E.= standard error

Table 3. Population parameters of *L. orbonalis* reared on different diets

Particulars	Potato Diet (PD)	Modified Artificial Diet (AD)
Net Reproductive Rate (R_0)	23.57	15.36
Finite Rate of Increase (λ)	1.12	1.10
Intrinsic Rate of Increase (R_m)	0.11	0.09
Mean Generation Time (T)	26.40	28.13
Gross Reproduction Rate (GRR)	39.25	32.13
Doubling Time (DT)	5.79	7.13

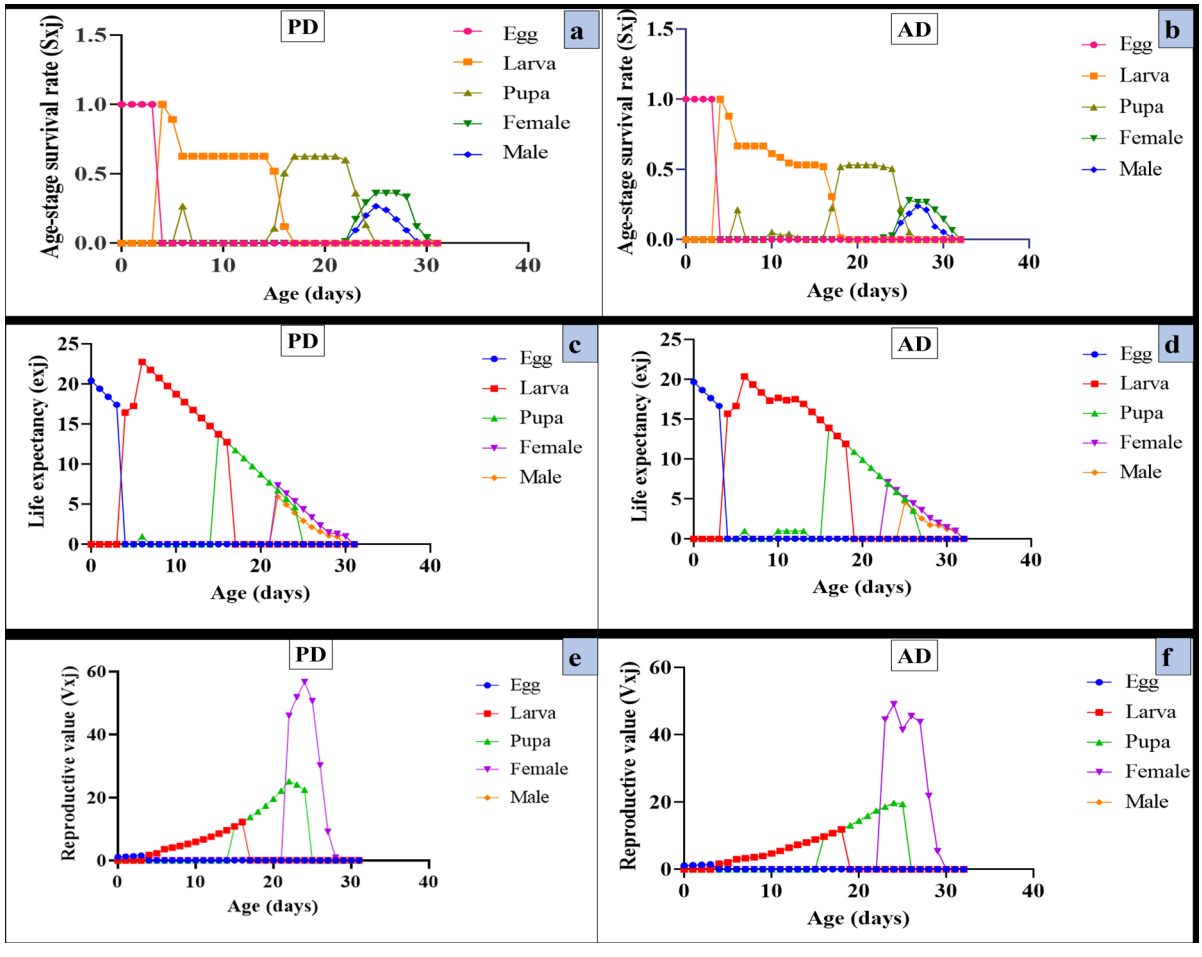


Figure 3. **(a)** and **(b)** Age-stage survival rate; **(c)** and **(d)** Stage-specific life expectancy; **(e)** and **(f)** Age-stage specific reproductive value of *L. orbonalis* reared on Potato Diet (PD) and Artificial Diet (AD), respectively.

Our results showed that, in PD, 62.6% of individuals survived to adult stage, whereas in AD 53.3% of individuals reached the adult stage. The mean larval and preadult duration was approximately 1.33 and 2 days longer, respectively in the case of AD than that in PD. There was no significant difference observed in the pupal duration between both diets (Table 2). The probability of stage-specific survival rate of larval and pupal stages was 0.89 and 0.70 in PD, while it was 0.88 and 0.60 in AD. Pre-adult survival rate (Sa) was higher in PD (0.62) than in AD (0.52).

The females reared on PD had higher total adult longevity, fecundity, and number of oviposition days than those reared on AD (Table 2). The age-stage-specific survival rate (Sxj), life expectancy (Exj) and age-stage reproductive value (Vxj) show better survival and multiplication of *L. orbonalis* on PD (Figure 3). The net reproductive rate (R_0) was comparatively higher in PD-reared individuals. The mean generation time (T) as well as doubling time (DT) was higher in AD-reared than in PD-reared insects (Table 3). In a study conducted by Islam *et al*., 2020 on *L. orbonalis* biology reported that the net reproductive rate, finite rate of increase, mean generation time and gross reproduction rate were 24.22, 1.15, 23.41 and 46.44, respectively when reared on the natural diet at 28ºC.

We have also observed the total developmental period (females) from egg to adult in PD was 23.67 days whereas in AD it was 25.23 days. Mannan *et al* (2015), observed that the total developmental period ranged from 21.18 days (summer) to 31.95 days (winter). Rice, 2007 reported it was 22 to 27 days whereas, it took 28.85 days according to Gupta and Kauntey (2007). The eggs hatched in 4 days. The larval duration observed in PD was 12.11 days whereas in AD was 13.44 days. Other studies reported a larval duration of 15-20days (Talekar *et al*., 1999), 18.66 days (Singh & Singh, 2001), 16.32 days (Kavitha *et al*., 2008) and 12.80 days (Gupta & Kauntey, 2007). The pupal duration in both diets was almost similar. In a similar type of study by Rahman *et al*. (2011) it was observed that the larval duration on potatoes was 13.1 days whereas on an artificial diet was 14.9 days. They have reported that the pupal duration was 8.17 days and 10.23 days in PD and AD, respectively. The pupal period for potato reared was 6.60 (summer) to 10.65 days (winter) (Mannan *et al*., 2015).

The female adult longevity recorded was 5.0 to 5.7 days among both diets. The female adult longevity reported in similar studies on *L. orbonalis* was 5.80 to 7.50 days (Kavitha *et al*., 2008; Mannan *et al*., 2015; Singh & Singh, 2001).We have observed a sex ratio of 1.33:1 and 1.12:1 in PD and AD, respectively. The other studies on *L. orbonalis* biology reported a sex ratio of 1.16:1 to 1.3:1 (Patial *et al*., 2007; Singla *et al*., 2018). The fecundity was 38 to 74.60 during different months of the year (Kavitha *et al*., 2008). The number of eggs laid ranged between 80 to 253 per female (Alam *et al*., 2003). We have also observed a fecundity of 52 and 65 eggs per female in AD and PD, respectively.

dsRNA stability in different diets

To induce a successful RNA interference (RNAi), the dsRNA must persist in a stable form for an ample period both in the diet provided and till it reaches the insect gut to allow

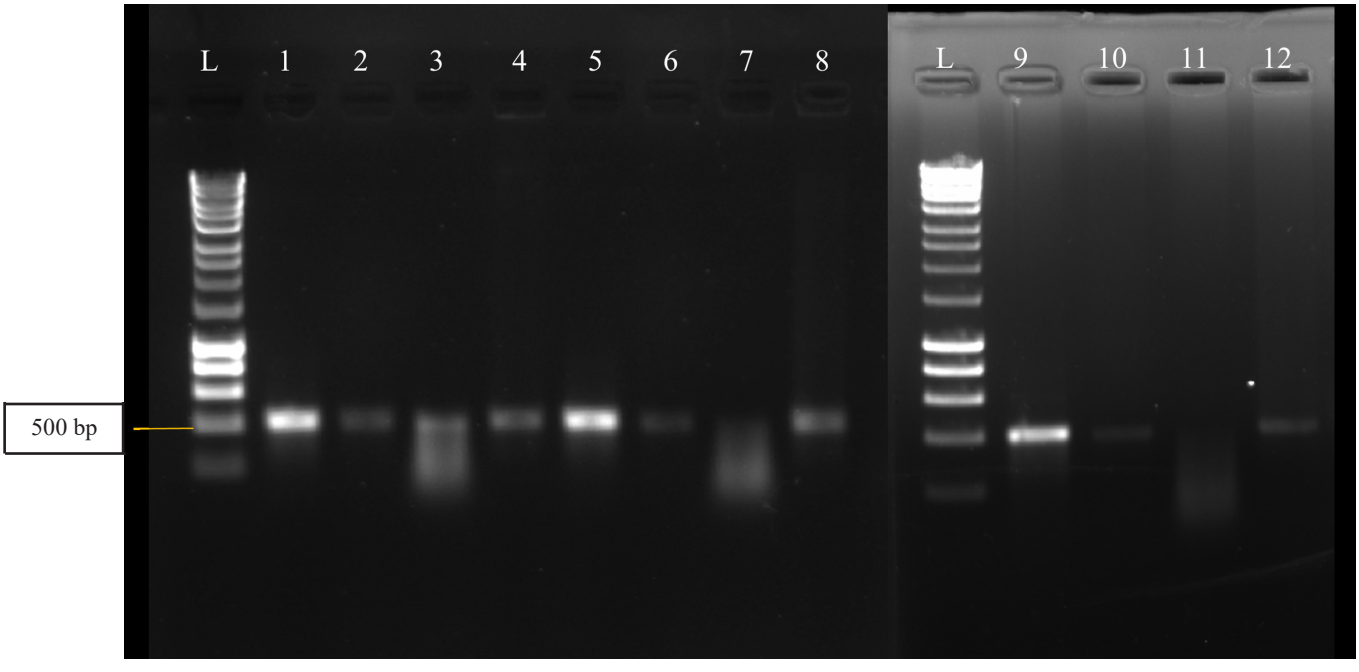


Figure 4. dsRNA stability in different diets at 6, 12, and 24 h post mixing. L- 1 kb ladder; 1, 5 and 9 show dsRNA in control; 2, 6 and 10 show dsRNA in brinjal; 3, 7 and 11 show dsRNA in potato; 4, 8 and 12 show dsRNA in AD, respectively at 6, 12 and 24 h post mixing diet with dsRNA.

internalization.

The dsRNA mixed with AD, PD, brinjal and molecular grade water (control) was checked for its stability on 1.2 per cent Agarose gel at 6, 12 and 24 h post mixing. We have observed that the dsRNA in molecular grade water was stable at all time points studied. On the other hand, the dsRNA in PD and brinjal diets started degrading at 12 h. and 24 h. post mixing respectively. The dsRNA in AD was found to be stable at 24 h post mixing but there was a reduction in the quantity, as noticeable by band intensity on the gel (Figure 4). A similar type of study conducted for dsRNA stability showed that dsRNA was stable for 6 days in an artificial diet during the assay for *Diaphorina citri* (Galdeano *et al*., 2017). Similarly, the artificial diets of *B. dorsalis*, *Nilaparvata lugens* (Li *et al*., 2011; Li *et al*., 2011) and *Bacterocera cockerelli* (Wuriyanghan & Falk, 2013), having V-ATPase dsRNA showed stable dsRNA during 6, 24 and 96 h post-feeding, respectively. In a study conducted to know the dsRNA stability in artificial diets of *Acyrthosiphon pisum* and *Tribolium castaneum*, it was found that there was a reduction in levels of intact dsRNA from 48 to 72 h post mixing in *A. pisum* diet but the dsRNA was detectable in *T. cataneum* diet up to 14 days (Cao *et al*., 2018). From our study, it can be suggested that the AD in which dsRNA was found stable for 24 h post mixing, can be used for the bioassay in *L. orbonalis*.

CONCLUSION

The dsRNA-based gene silencing, the next-generation pest management tool can ensure an ecologically responsible way of tackling the pest problems. Efficient delivery of dsRNA into the insect system through an approach similar to field application can give a more reliable result in the bioassays. Our study evaluated the potential of different diets regularly used in bioassays of *L. orbonalis* for supporting insect biology and efficient delivery of dsRNA. We came out with a result that, the modified artificial diet (AD), could be used for dsRNA-based bioassays. The potato diet (PD) which had shown better results for insect biology parameters can be used for insect rearing.

ACKNOWLEDGEMENT

The authors are highly grateful for the support of the Director ICAR-NBAIR, Bangalore, India. The first author of the manuscript is also thankful for the support from Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh.

REFERENCES

Alam, S. N., Rashid, M. A., Rouf, F. M. A., Jhala, R. C., Patel, J. R., Satpathy, S., … Cork, A. 2003. Development of an integrated pest management strategy for eggplant fruit and shoot borer in South Asia. AVRDC-WorldVegetableCenter.

Bolognesi, R., Ramaseshadri, P., Anderson, J., Bachman, P., Clinton, W., Flannagan, R., … Moar, W. 2012. Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm *(Diabrotica virgifera virgifera* LeConte). *Plos One*, **7**(10): Article e47534. https://doi.org/10.1371/journal.pone.0047534 PMid:23071820 PMCid:PMC3469495

Boopal, K., Arya, V. V, Rami Reddy, P. V, Vageeshbabu, and Hanur, S. 2013. Potato a suitable alternative host for brinjal fruit shoot and fruit borer *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae). *Int J Agric Sci,* **3**.

Cao, M., Gatehouse, J. A., and Fitches, E. C. 2018. A systematic study of RNAi effects and dsRNA stability in *Tribolium castaneum* and *Acyrthosiphon pisum*, following injection and ingestion of analogous dsRNAs. *Int J Mol Sci*, **19**(4): Article 1079. https://doi.org/10.3390/ijms19041079 PMid:29617308 PMCid:PMC5979293

Chi, H. 1988. Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ Entomol*, **17**(1): 26-34. https://doi.org/10.1093/ee/17.1.26

Chi, H. 2014. TWOSEX-MSChart: A computer program for the age-stage, two-sex life table analysis .Available online: http://140.120.197.173/Ecology/prod02.htm

Chi, H., and Liu, H. 1985. Two new methods for the study of insect population ecology. *Bull Inst Zool Acad Sin*, **24**(2): 225-240.

Christiaens, O., Swevers, L., and Smagghe, G. 2014. DsRNA degradation in the pea aphid (*Acyrthosiphon pisum*) associated with lack of response in RNAi feeding and injection assay. *Peptides*, **53**: 307-314. https://doi.org/10.1016/j.peptides.2013.12.014 PMid:24394433

Elbashir, S. M., Martinez, J., Patkaniowska, A., Lendeckel, W., and Tuschl, T. 2001. Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *EMBO J*, **20**(23): 6877-6888. https://doi.org/10.1093/emboj/20.23.6877 PMid:11726523 PMCid:PMC125328

Galdeano, D. M., Breton, M. C., Lopes, J. R. S., Falk, B. W., and Machado, M. A. 2017. Oral delivery of double-stranded RNAs induces mortality in nymphs and adults of the Asian citrus psyllid, *Diaphorina citri*. *PLoS One*, **12**(3): Article e0171847. https://doi.org/10.1371/journal.pone.0171847 PMid:28282380 PMCid:PMC5345766

Gupta, Y. C., and Kauntey, R. P. S. 2007. Biology of shoot and fruit borer, *Leucinodes orbonalis* Guenee of brinjal, *Solanum melongena* Linn. at Mathura, Uttar Pradesh, India. *Asian J Exp Sci*, **21**(1): 155-160.

Islam, T., Roknuzzaman, A. H. M., Hassan, K., and Ullah, M. S. 2020. Temperature impacts on the eggplant shoot and fruit borer *Leucinodes orbonalis*: A life table approach. *Int J Trop Insect Sci*, **40**: 351-360. https://doi.org/10.1007/s42690-019-00086-7

Jing, Y., and Han, Z.-J. 2014. Efficiency of different methods for dsRNA delivery in cotton bollworm (*Helicoverpa armigera*). *J Integr Agric*, **13**(1): 115-123. https://doi.org/10.1016/S2095-3119(13)60511-0

Kariyanna, B., Prabhuraj, A., Mohan, M., Bheemanna, M., Basavaraj Kalmath, P. Y., and Diwan, J. R. 2020. Insecticide usage pattern and evolution of resistance in eggplant shoot and fruit borer, *Leucinodes orbonalis* guenée (Lepidoptera: Crambidae) in India. *Plant Arch*, **20**(2): 1255-1261.

Kavitha, V. S., Revathi, N., and Kingsley, S. 2008. Biology of brinjal pest, *Leucinodes orbonalis* Guen. of Erode region in Tamil Nadu. *J Entomol Res*, **32**(3): 255-257.

Kodandaram, M. H., Rai, A. B., Sharma, S. K., and Singh, B. 2017. Shift in the level of susceptibility and relative resistance of brinjal shoot and fruit borer *Leucinodes orbonalis* (Guen) to diamide insecticides. *Phytoparasitica*, **45**(2): 151-154. https://doi.org/10.1007/s12600-017-0584-z

Li, J., Chen, Q., Lin, Y., Jiang, T., Wu, G., and Hua, H. 2011. RNA interference in *Nilaparvata lugens* (Homoptera: Delphacidae) based on dsRNA ingestion. *Pest Manag Sci*, **67**(7): 852-859. https://doi.org/10.1002/ps.2124 PMid:21370391

Li, X., Zhang, M., and Zhang, H. 2011. RNA interference of four genes in adult *Bactrocera dorsalis* by feeding their dsRNAs. *PloS One*, **6**(3): Article e17788. https://doi.org/10.1371/journal.pone.0017788 PMid:21445257 PMCid:PMC3060817

Mally, R., Korycinska, A., Agassiz, D. J. L., Hall, J., Hodgetts, J., and Nuss, M. 2015. Discovery of an unknown diversity of Leucinodes species damaging Solanaceae fruits in sub-Saharan Africa and moving in trade (Insecta, Lepidoptera, Pyraloidea). *ZooKeys*, **472**: Article 117. https://doi.org/10.3897/zookeys.472.8781 PMid:25632252 PMCid:PMC4304033

Mannan, M. A., Islam, K. S., Jahan, M., and Tarannum, N. 2015. Some biological parameters of brinjal shoot and fruit borer, *Leucinodes orbonalis* guenee (Lepidoptera: Pyralidae) on potato in laboratory condition. *Bangladesh J Agril Res*, **40**(3): 381-390. https://doi.org/10.3329/bjar.v40i3.25412

Muhammad, W., Javed, K., Javed, H., Ahmad, M., and Azam Khan, M. 2020. The impact of transgenic brinjal for the control of *Leucinodes orbonalis* Guenee and possible effects on non-target arthropods: a comprehensive review. *Plant Prot*, **4**(3): 131-135. https://doi.org/10.33804/pp.004.03.3420

Patial, A., Mehta, P. K., and Sood, A. K. 2007. Developmental biology of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee in mid-hills of Himachal Pradesh. *Entomon*, **32**(2): Article 137.

Prodhan, M. Z. H., Hasan, M. T., Chowdhury, M. M. I., Alam, M. S., Rahman, M. L., Azad, A. K., … Shelton, A. M. 2018. Bt eggplant (*Solanum melongena* L.) in Bangladesh: Fruit production and control of eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenee), effects on non-target arthropods and economic returns. *PLoS One*, **13**(11): Article e0205713. https://doi.org/10.1371/journal.pone.0205713 PMid:30462653 PMCid:PMC6248903

Rahman, M. M., Mahmud, M. T. M., Hasan, M. F., Habib, S. H., and Kausar, H. 2011. Study on the growth and development of brinjal shoot and fruit borer with different diets. *Afr J Biotechnol*, **10**(57): 12299-12302. https://doi.org/10.5897/AJB10.1428

Rice, P. 2007. Integrated pest management in rice-vegetable cropping systems. *Nueva Ecija, Maligaya: Science City of Munoz*, **73**.

Singh, Y. P., and Singh, P. P. 2001. Biology of shoot and fruit borer (*Leucinodes orbonalis* Guen.) of egg plant (*Solanum melongena* L.) at medium high altitude hills of Meghalaya. *Indian Journal of Entomology*, **63**(4): 373.

Singla, P., Bhullar, M. B., and Kaur, P. 2018. Biological studies on brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee. *J Entomol Zool Stud*, **6**(1): 161-165.

Talekar, N. S., Lin, M. Y., and Hwang, C. C. 1999. Rearing eggplant fruit and shoot borer: A slide set and illustrated guide. Shanhua, Tainan, AVRDC.

Tian, H., Peng, H., Yao, Q., Chen, H., Xie, Q., Tang, B., and Zhang, W. 2009. Developmental control of a lepidopteran pest *Spodoptera exigua* by ingestion of bacteria expressing dsRNA of a non-midgut gene. *PloS One*,

4(7): Article e6225. https://doi.org/10.1371/journal.pone.0006225 PMid:19593438 PMCid:PMC2704864

Willkomm, S., Zander, A., Grohmann, D., and Restle, T. 2016. Mechanistic insights into archaeal and human argonaute substrate binding and cleavage properties. *PloS One*, **11**(10): Article e0164695. https://doi.org/10.1371/journal.pone.0164695 PMid:27741323 PMCid:PMC5065165

Wuriyanghan, H., and Falk, B. W. 2013. RNA interference towards the potato psyllid, *Bactericera cockerelli*, is induced in plants infected with recombinant tobacco mosaic virus (TMV). *PloS One*, **8**(6): Article e66050. https://doi.org/10.1371/journal.pone.0066050 PMid:23824081 PMCid:PMC3688868