Pesticidal Potentials Of Some Red Algal Seaweeds From Tuticorin Coast Against The Tobacco Cutworm *Spodoptera Litura* Fab. (Lepidoptera: Noctuidae)

Gowthish K, Kannan R

Abstract:In this study we observed the effect of four red algal seaweed's extract using methanol solvent on various life stages of *S.litura* such as larval mortality, pre-pupal mortality, pupation, pupal malformation and larval to adult conversion ratio and the results are presented in both table and graphical form. The causes of all these seaweeds on larvae begin from 48hrs except *H. valentiae* which produced mortality after 60hrs of treatment. The highest larvicidal activity (53.33%) was observed after 72 hrs of treatment at the maximum concentration 10 per cent by the *G. edulis* whereas *G. folifera* and *G. lithophila* were produced similar larvicidal activity (33.33%) and least larval dead was counted in *H. valentiae* at 26.66 per cent larval mortality respectively.

Index Terms:Red algal seaweeds, insecticidal activity, methanol solvent extract, *Spodoptera litura.*

1 INTRODUCTION

The tobacco cutworm *Spodoptera litura* Fab. is one of the important insect pest of nearly 120 plant species in cultivated crops of both agricultural and horticultural ecosystem distributed tropical and subtropical region of Asian continent. They categorized as major pest based on their wide host range, abundance and damage on host plants. The larval stage is most destructive stage and due to their gregarious feeding habit they makes heavy damage on various parts of crop plants Eg. Leaves, flowers, fruits, etc., with accountable yield losses in short period of time (Ahmad *et al.,* 2013). So for, Chemical insecticides have recommended for managing these insects but the repeated and excessive dose than recommended leads to resistance development against the commonly used insecticide groups such as, organochlorine, organophosphates, carbamates, pyrethroids (Ahmad *et al.,* 2007; Saleem *et al.,* 2008) and newer insecticides like indoxacarb, abamectin, avermectin, fipronil and methoxyfenozide (Ahmad *et al.,* 2015; Ahmad and Mehmood, 2015; Ahmad and Gull, 2017). To overcome, these problems alternate management practices need to be follow and as the vast availability of natural resources in the marine ecosystem especially from marine algae may be a right choice because the presence of abundant quantity of secondary metabolites. Many studies already have been reported the presence of chemical constituent in seaweeds that act against the biology of insects *Viz*, Brown - *Dictyota dichotoma* against *Aedes aegypti* (Thangam and Kathiresan, 1991), *Padina pavonica* against *Culex pipiens* (Elbanna and Hegazi, 2011), *P. pavonica*, *Sargassum tenerrimum* against *Dysdercus cingulatus* (Sahayaraj and Kalidas, 2011; Sahayaraj and Mary Jeeva, 2012), *S. dentifolium* against *Spodoptera littoralis* and *S. frugiperda* (Matloub *et al.,* 2012), *S. cristaefolium* against *S. litura* (Gowthish and Kannan, 2018). Green - *Caulerpa scalpelliformis* against *A. aegypti* (Thangam and Kathiresan, 1991), *Ulva fasciata* against *Culex* sp. (Selvin and Lipton, 2004), *C. scalpelliformis* against *S. litura* and *D. cingulatus* (Kombiah and Sahayaraj, 2012), *C. racemosa* against *A. aegypti, C. quinquefasciatus* and *Anopheles stephensi* (Ali *et al.,* 2013). Red - *Hypnea musciformis* against *Culex* sp. (Selvin and Lipton, 2004), *Lobophora variegata* against *A. aegypti* (Manilal *et al.,* 2011), *Laurencia dendroidea* and *H. musciformis* against *A. aegypti* (Bianco *et al.,* 2013), *Bryopsis pennata* against *A. aegypti* (Xin Yu *et al.,* 2015), *Laurencia intricata* against *Sitophilus zeamais* (Ishii *et al.,* 2018), *Liagora ceranoides* against *Spodoptera litura* (Kannan and Dharani Priya, 2019). So, the objective of this study is to evaluate insecticidal activity of certain red algal seaweeds collected from the coastal line of Tuticorin Sea under *in vitro* condition.

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2 MATERIALS AND METHODS

2.1 Collection of Macro algae from Tuticorin coast

The four red algal seaweeds namely, *Gracilaria edulis, G. folifera, Grateloupia lithophila* and *Hypnea valentiae* (Fig. 1 to 4) were collected from rocks of subtidal region in Hare Island, Tuticorin, Tamil Nadu, India by hand picking method and were washed with seawater and three times repeatedly washed with tap water to eliminate the excess salt, sand and epiphytes. Then, the algae were spread on newspapers for shade dry at fortnight interval to safe storage (Kannan and Bharathkumar, 2016). The seaweeds were identified at CAS Marine Biology, Annamalai University.

2.2 Mass culturing of Test insects

The *Spodoptera litura* egg masses were collected from black gram field at Sivapuri village in Cuddalore district of Tamil Nadu. The field collected egg masses were allowed to hatch under laboratory condition (25±2□C) temperature and (75±5%) Relative humidity. Newly hatched young ones were fed with fresh castor leaves (*Ricinus communis* L.) on regular basis throughout the larval period. For pupation, soil was provided in a sterilized plastic tray (formalin 1%). After

pupation, the pupae were placed inside the ovipositional cage (40 x 25 x 25 cm). The emerged adult moths (five pairs) were fed with 10% honey solution and allowed for mating and for egg laying Nerium leaves were kept inside ovipositional cage. After hatching, the neonates were fed with fresh tender castor leaves and were reared up to third instar stage in plastic trays. The uniform aged third instar larvae were used for bioassay experiment.

2.3 Preparation of solvent extract

Each of the seaweeds were (30gms) partially powdered, packed and loaded separately in Soxhlet apparatus (GI-1706 A) and refluxed with 300ml of methanol solvent for 24 hours continuously. The extracted each solvent extract were transferred into 500ml beaker and evaporated on a hot plate. Extracted crude were used for the evaluation experiments. The extracts were stored at -200C (Kombiah and Sahayaraj, 2012).

2.4 Bioassay – free choice method

The crude extract 10gms were diluted in 100ml of methanol solvent for preparing 10 per cent stock solution. The solvent extracts at 0.5, 1.00, 2.00, 4.00, 6.00, 8.00 and 10.00 per cent concentration were prepared along with the absolute control and standard check. The third instar homogenous population of S. litura larvae were used. For leaf dipping method, 5cm dia castor leaf discs were prepared, surface sterilized and dipped in solvent extract for 10 minutes and air dried. The five numbers of treated leaf discs were placed in Petri plate and three hours pre-starved larva were introduced in each Petri plate according to the treatment schedule and allowed to feed on the treated leaves. The experiment was performed under completely randomized design (Nine treatments with three replications). Data on larval mortality, pre-pupal mortality, pupation, pupal malformation and adult emergence were collected and the means were pooled and analysed statistically were presented.

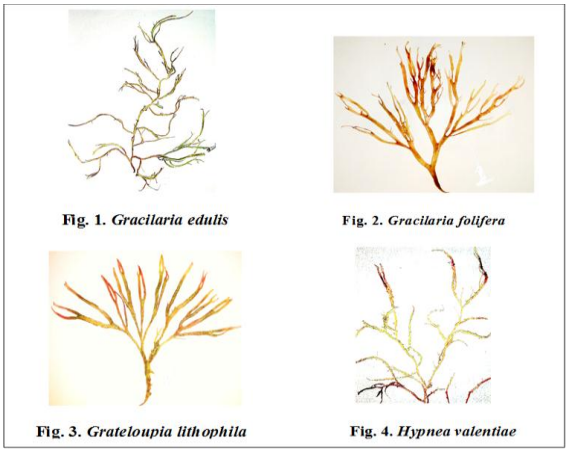

Fig. 1. *Gracilaria edulis*

Fig. 2. *Gracilaria folifera*

Fig. 3. *Grateloupia lithophila*

Fig. 4. *Hypnea valentiae*

3 RESULTS AND DISCUSSIONS

In this study we observed the effect of four red algal seaweed's extract using methanol solvent on various life stages of *S.litura* such as larval mortality, pre-pupal mortality, pupation, pupal malformation and larval to adult conversion ratio and the results are presented in both table and graphical form as follows. The impact of these seaweeds were observed from larval stage to adult stage. The causes of all these seaweeds on larvae begin from 48hrs except *H. valentiae* which produced mortality after 60hrs of treatment. The highest larvicidal activity (53.33%) was observed after 72 hrs of treatment at the maximum concentration 10 per cent by the *G. edulis* whereas *G. folifera* and *G. lithophila* were produced similar larvicidal activity (33.33%) and least larval dead was counted in *H. valentiae* at 26.66 per cent larval mortality respectively. Before getting pupation the countable number of larvae got shrink and unable to moult their skin which later became inactive and their pre-pupal dead was observed in all the four seaweed extracts and result had been confirmed the pre-pupal action against test insects. Among them *G. edulis* posed maximum pre-pupal death (26.66%) compared to other three seaweeds wherein they shown 13.33 per cent pre-pupal mortality at 10 per cent concentration (Table 1). All the treatment had remaining larvae and the leftover larvae got pupated where the least pupation was observed in the *G. edulis* at pupation rate of 20 per cent and it was observed in *G. folifera* and *G. lithophila* as 53.33 per cent and highest pupation observed in *H. valentiae* at 60 per cent respectively (Fig. 1). We observed that even pupated test insects were got partially malformed and unable to form complete pupal structure and as the result they fail to became adult and the data exhibited that the highest pupal malformation (20.00%) was seen in *G. edulis* followed by *G. lithophila* demonstrated 13.33 per cent whereas *G. folifera* and *H. valentiae* produced least pupal dead (6.66%) at maximum test concentration of the treatment (Table 1). Among the four seaweeds, *G. edulis* produced high impact on *S. litura* wherein no adult emerged in the *G. edulis* treatments whereas *G. lithophila* tested treatments showed an adult emergence of 40.00 per cent followed by 46.66 and 53.33 per cent by *G. folifera* and *H. valentiae* respectively and throughout the treatment period no larval, pre-pupal, pupal malformation was observed in both absolute control and solvent control which resulted in 100 per cent adult emergence respectively (Fig. 2). Larval to adult conversion was worked out to understand the susceptible stage of test insects especially their insect growth regulatory activity in each stages of insects and to ascertain the number of insects released and emerged out from treatment check (Table 2).

3.1 Discussion

Our study of methanol extract of these four seaweeds confirmed that the presence of insecticidal action with significant variation between the treatments and all the seaweeds were tested in this experiment produced accountable insecticidal action at various life stages of test insects such as larval mortality, reduced pupation, pre-pupal mortality and reduced adult emergence and our results are somewhat similar to the following studies on the efficacy of seaweed extract against variety of insects work done by many researchers in India and abroad. The strong insect repellent activity of maize weevil *Sitophilus zeamais* was observed from the compound, Laurinterol (Ishii *et al.,* 2017) and Cyclocolorenone (*Ishii et al.,* 2018) which are isolated from the red algae *Laurencia nidifica* and *L. intricata* respectively. The result observed on the egg mortality, reduction in adult fertility and population of a stored grain pest, *Callosobruchus maculates* when the grains are treated with hot ethanolic extract of Green macro alga, *Cladophora glumerata* the extracts were found to contain Saponins, Tannins, Alkaloids, Flavonoids and phenols (Mohammed *et al.,* 2018). The mechanism of action of Saponins was blocking the uptake of

sterols that led to decreased reproduction and increased mortality in insect pests because the insects unable to produce sterol by themselves (Belled *et al.,* 2005) and it also have antifeedent antimicrobial, anti-inflammatory and hemolytic effects (Xu *et al.,* 1996; Francis *et al.,* 2002) The aqueous extract of *Hypnea musciformis* was potent against larva, pupa, longevity and fecundity of *Plutella xyllostella* (Roni *et al.,* 2015). The insecticidal activity of *Sargassum cristafolium* (Gowthish and Kannan, 2018); *Acanthopora spicifera* (Dharanipriya and Kannan, 2018) and *Liagora ceranoides* (Kannan and Dharani priya, 2019) were reported against *Spodoptera litura*. The benzene extracts of *Padina pavonica* (Sahayaraj and Kalidas, 2011); methanol extract of green algae *Ulva fasciata* and *U. lactuca* Asha *et al.* (2012); and the chloroform extract of *S. weightii* and *P. pavonica* (Asaraja and sahayaraj,2013) marked nymphicidal and ovicidal action.

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REFERENCES

[1] *Ahmad, M and R. Mehmood. 2015. Monitoring of resistance to new chemistry insecticides in Spodoptera litura (Lepidoptera: Noctuidae) in Pakistan. Journal of Economic Entomology, 108: 1279–1288.*

[2] *Ahmad, M and S. Gull. 2017. Susceptibility of armyworm Spodoptera litura (Lepidoptera: Noctuidae) to novel insecticides in Pakistan. Can. Entomol. 00: 1-13*

[3] *Ahmad, M., A. H. Sayyed, M. A. Saleem and M. Ahmad. 2008. Evidence for field evolved resistance to newer insecticides in Spodoptera litura (Lepidoptera: Noctuidae) from Pakistan. Crop Prot. 27: 1367–1372.*

[4] *Ahmad, M., M. I. Arif and M. Ahmad. 2007. Occurrence of insecticide resistance in field populations of Spodoptera litura (Lepidoptera: Noctuidae) in Pakistan. Crop Prot. 26: 809–817.*

[5] *Ali, M.Y.S., S. Ravikumar and J.M. Beula. 2013. Mosquito larvicidal activity of seaweeds extracts against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. Asia. Pac. J. Trop. Dis. 3: 196 – 201*

[6] *Asaraja, A and Sahayaraj, K. 2013. Screening of insecticidal activity of brown macroalgal extracts against Dysdercus cingulatus (Fab.) (Hemiptera: Pyrrhocoridae). Journal of Biopesticides. 6(2):193-203.*

[7] *Asha, A., Rathi, J.M., Raja, D.P and Sahayaraj, K. 2012. Biocidal activity of two marine green algal extracts against third instar nymph of Dysdercus cingulatus (Fab.) (Hemiptera: Pyrrhocoridae). Journal of Biopesticides. 5: 129-134.*

[8] *Belled, X., Martin, D and Piulachs, M.D. 2005. The mevalonate pathway and the synthesis of juvenile hormone in insects. Annual Review of Entomology. 50:181-199.*

[9] *Bianco, E.M., L. Pires, G.K.N. Santos, K.A. Dutra, T.N.V. Reis, E.R.T.P.P. Vasconcelos, A.L.M. Cocentino and D.M.A.F. Navarro. 2013. Larvicidal activity of seaweeds from northeastern Brazil and of a halogenated sesquiterpene against the dengue mosquito (Aedes aegypti). Ind. Crop. Prod. 43: 270 - 275. https://doi.org/10.1016/j.indcrop.2012.07.032.*

[10] *Elbanna S M and M. M. Hegazi. 2011. Screening of some seaweeds species from South Sinai, Red Sea as potential bioinsecticides against mosquito larvae; Culex pipiens. Egypt. Acad. J. biolog. Sci. 4 (2): 21-30.*

[11] *Francis, G., Kerem, Z., Makkar, H. P and Becker, K. 2002. The Biological Action of Saponins in Animal Systems: A Review. British Journal of Nutrition. 88(6): 587-605.*

[12] *Gowthish, K and R. Kannan. 2018. Bioefficacy of brown algal seaweed Sargassum cristaefolium C. Agardh against a cosmopolitan pest, Spodoptera litura Fabricius (Lepidoptera: Noctuidae). Multilogic in Science. VIII (D): 56 – 57 https://doi.org/10.1016/S2222-1808(13)60040-7.*

[13] *Ishii T, Shinjo Y, Miyagi M, Matsuura H, Abe T, Kikuchi N and Suzuki M. 2018. Investigation of insect repellent activity of cyclocolorenone obtained from the Red alga Laurencia intricata. Rec. Nat. Prod. 10 (10): 20*

[14] *Ishii, T., Nagamine, T. Nguyen, B. C. Q and Tawata. S. 2017. Insecticidal and repellent activities of laurinterol from the Okinawan red alga Laurencia nidifica. Rec. Nat. Prod. 11: 63-68.*

[15] *Kannan, R and N. Dharani Priya. 2019. Studies on Methanolic Extract of Brown Algal Seaweed Liagora ceranoides J.V. Lamouroux from Southern Coast of Tamil Nadu: In vitro Anti-Insect Properties and Phytochemicals. Nat. Prod. Chem.S Res.7 (1): 354*

[16] *Manilal, A., N. Thajuddin, J. Selvin, A. Idhayadhul, R.S. Kumar and S. Sujith. 2011. In vitro mosquito larvicidal activity of marine algae against the human vectors, Culex quinquefasciatus (Say) and Aedes aegypti (Linnaeus) (Diptera: Culicidae). Int. J. Zool. Res. 7: 272 - 278.*

[17] *Matlouba, A. A., N. E. Awad and O. A. Khamiss. 2012. Chemical composition of some Sargassum spp. and their insecticidal evaluation on nucleopolyhedrovirus replication in vitro and in vivo. Egyp. Pharm. J. 11:53–58*

[18] *Mohammed, D.Y., Mahmood, S.I and Dwaish, A.S. 2018. The effect of hot ethanolic algae extract of Cladophora glumerata on some biological aspect of Callosobruchus maculates (Fab). Journal of pharmaceutical sciences and research 10(3): 532-536.*

[19] *Roni, M., Murugan, K., Panneerselvam, C., Subramaniam, J., Nicoletti, M., Madhiyazhagan, P., Dinesh, D., Suresh, U., Khater, H.F., Wei, H., Canale, A., Alarfaj, A.A., Munusamy, M.A., Higuchi, A and Benelli, G. 2015. Characterization and biotoxicity of Hypnea musciformis-synthesized silver nanoparticles as potential eco-friendly control tool against Aedes aegypti and Plutella xylostella. Ecotoxicology and Environmental Safety. 121: 31–38.*

[20] *Sahayaraj, K., and S. Kalidas. 2011. Evaluation of nymphicidal and ovicidal effect of seaweed, Padina pavonica (Linn.) (Phaeophyceae) on cotton pest, Dysdercus cingulatus (Fab.). Indian Journal of Marine Sciences 40:125-129*

[21] *Saleem, M. A., M. Ahmad, M. Ahmad, M. Aslam and A. H. Sayyed. 2008. Resistance to selected organochlorine, organophosphates, carbamates and pyrethroids in Spodoptera litura (Lepidoptera: Noctuidae) from Pakistan. J. Econ. Entomol. 101: 1667–1675.*

[22] *Selvin, J and A. P. Lipton. 2004. Biopotentials of Ulva fasciata and Hypnea musciformis collected from the*

peninsular coast of India. J. Marine Sci. and Tech. 12 (1):1-6

[23] Thangam, T.S and K. Kathiresan. 1991. Mosquito Larvicidal Activity of Marine Plant Extracts with Synthetic Insecticides. Bot. Mar.34 (6): 537-539.

[24] Xin Yu, Lee Wong C, Ahmad R, and Jantan I. 2015. Larvicidal activity, inhibition effect on development, histopathological alteration and morphological aberration induced by seaweed extracts in Aedes aegypti (Diptera: Culicidae), Asian Pacific Journal of Tropical Medicine. http://dx.doi.org/10.1016/j.apjtm.2015.11.011

[25] Xu, R., Zhao, W., Xu, J., Shao, B and Qin, G. 1996. Studies on bioactive saponins from chinese medicinal plants saponins used. Traditional and Modern Medicine. 2(6): 371-382.

Table 1. Insecticidal Activity of Red Algal Seaweeds

SEAWEEEDS	T_1 (0.5%)	T_2 (1%)	T_3 (2%)	T_4 (4%)	T_5 (6%)	T_6 (8%)	T_7 (10%)	Absolute control	Solvent control	SEd	CD (p =0.05)
Larval Mortality (%) (up to 72 hrs)											
Gracilaria edulis	20.00 (26.554)	26.66 (30.775)	33.33 (34.995)	33.33 (34.995)	40.00 (39.216)	46.66 (43.060)	53.33 (46.904)	0.00 (0.00)	0.00 (0.00)	**4.294**	**9.092**
Gracilaria folifera	6.66 (8.851)	13.33 (17.703)	20.00 (26.554)	20.00 (26.554)	26.66 (30.775)	26.66 (30.775)	33.33 (34.995)	0.00 (0.00)	0.00 (0.00)	**6.833**	**14.468**
Gratelopia lithophila	6.66 (8.851)	13.33 (17.703)	20.00 (26.554)	20.00 (26.554)	26.66 (30.775)	33.33 (34.995)	33.33 (34.995)	0.00 (0.00)	0.00 (0.00)	**6.833**	**14.468**
Hypnea valentiae	0.00 (0.00)	6.66 (8.851)	13.33 (17.703)	13.33 (17.703)	20.00 (26.554)	20.00 (26.554)	26.66 (30.775)	0.00 (0.00)	0.00 (0.00)	**7.496**	**15.871**
Pre-pupal Mortality (%) (72 - 96 hrs)											
Gracilaria edulis	6.66 (8.851)	6.66 (8.851)	6.66 (8.851)	13.33 (17.703)	20.00 (26.554)	20.00 (26.554)	26.66 (30.775)	0.00 (0.00)	0.00 (0.00)	**8.579**	**18.164**
Gracilaria folifera	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.66 (8.851)	6.66 (8.851)	6.66 (8.851)	13.33 (17.703)	0.00 (0.00)	0.00 (0.00)	**8.345**	**N/A**
Gratelopia lithophila	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.66 (8.851)	13.33 (17.703)	13.33 (17.703)	0.00 (0.00)	0.00 (0.00)	**7.227**	**N/A**
Hypnea valentiae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.66 (8.851)	6.66 (8.851)	6.66 (8.851)	13.33 (17.703)	0.00 (0.00)	0.00 (0.00)	**8.345**	**N/A**
Pupal malformation (%) (120 - 144hrs)											
Gracilaria edulis	6.66 (8.851)	6.66 (8.851)	13.33 (17.703)	13.33 (17.703)	20.00 (26.554)	20.00 (26.554)	20.00 (26.554)	0.00 (0.00)	0.00 (0.00)	**8.345**	**17.669**
Gracilaria folifera	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.66 (8.851)	6.66 (8.851)	6.66 (8.851)	0.00 (0.00)	0.00 (0.00)	**7.227**	**N/A**
Gratelopia lithophila	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.66 (8.851)	6.66 (8.851)	6.66 (8.851)	13.33 (17.703)	0.00 (0.00)	0.00 (0.00)	**8.345**	**N/A**
Hypnea valentiae	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.66 (8.851)	0.00 (0.00)	0.00 (0.00)	**4.173**	**N/A**

Table 2. Larval to Adult conversion ratio

L to A conversion ratio

Treatments	T_1 (0.5%)	T_2 (1%)	T_3 (2%)	T_4 (4%)	T_5 (6%)	T_6 (8%)	T_7 (10%)	Absolute control	Solvent control
Gracilaria edulis	1:0.66	1:0.60	1:0.46	1:0.40	1:0.20	1:0.13	1:0.00	1:1	1:1
Gracilaria folifera	1:0.93	1:0.86	1:0.80	1:0.73	1:0.60	1:0.60	1:0.46	1:1	1:1
Gratelopia lithophila	1:0.93	1:0.86	1:0.80	1:0.73	1:0.60	1:0.46	1:0.40	1:1	1:1
Hypnea valentiae	1:1	1:0.93	1:0.86	1:0.80	1:0.73	1:0.73	1:0.53	1:1	1:1

Fig. 1. Pupation Percentage

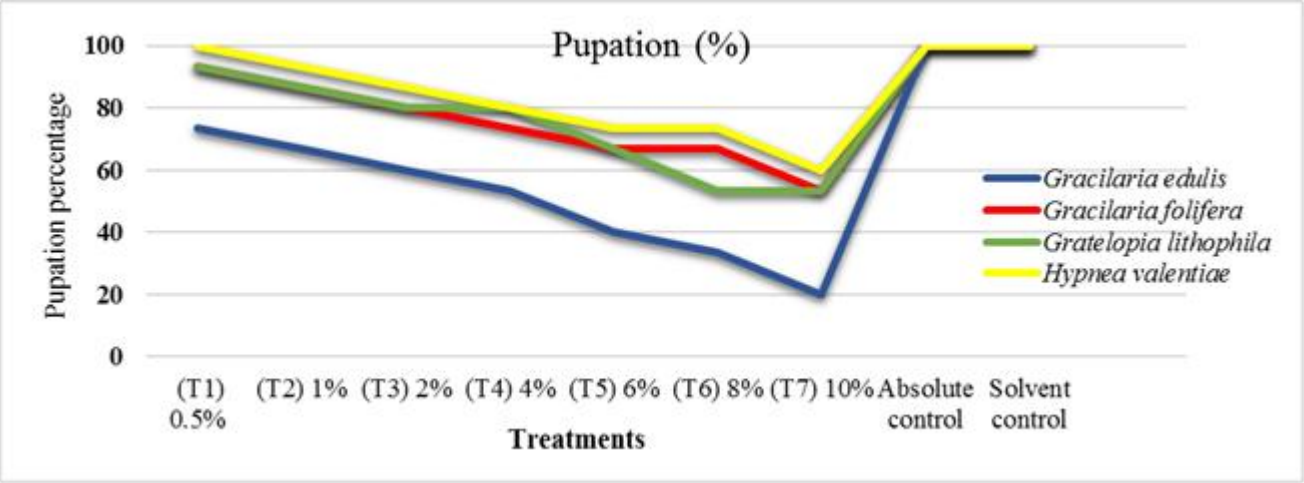

Fig.2. Adult Emergence Percentage