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Insecticidal activity of lectin from *Perinereis Cultrifera* against hibiscus mealybug

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Abstract
The present study dealt with the evaluation of the insecticidal activity of lectin purified from marine polychaetes collected from the Gadilam estuary against the mealybugs infestation in *Hibiscus* plants. The collected polychaete species was identified as *Perinereis cultrifera*. Lectin was extracted from the polychaete and it was designated as 'PcL'. The extracted PcL showed the highest hemagglutination titer of 32 HU (hemagglutination unit) against rabbit erythrocytes. Purification of the extracted crude lectin was done using a series of purification steps and 60% ammonium sulphate saturation results in highest protein precipitation of 22 mg/ml. This was furtuer purified using DEAE-Cellulose and DEAE- Sephadex A-50 column chromatography techniquies. The specific activity was found to be increased to 890.4 U/mg with 6.3 fold purity after the purification in DEAE-cellulose column whereas; it was 1280 U/mg with 9 fold purity after the DEAE-sephadex column chromatographic purification. The purified lectin was evaluated for its insecticidal activity against mealybugs infestation in *Hibiscus* plants. The results showed excellent insecticidal activity on a dose dependent manner with the highest insect mortality of 73.33% at 10 mg/ml of lectin concentration with the LC_{50} value of 6.8 mg/ml. The field study also showed effective insecticidal activity after 3 days of application.

Keywords: Lectin, polychaete, *Perinereis cultrifera*, mealybug, biocontrol, insecticidal activity

Introduction

Effective pest control for the protection of crops involves application of various chemical pesticides. However, the uncontrollable use of these synthetic insecticides causes many problems including environmental pollution and also some insecticides kill non-targeted beneficial insects (Colosio and Moretto, 2008) and eventually, their prolonged use against these insect pests cause development of resistance to these insecticides over a period of time (Li *et al*., 2007). Hence, entomologists aroud the globe are looking for an eco-friendly, bio-based alternative insecticides for the control of insect pests.

Polychaetes are the most common macrofauna inhabit the soft bottoms, intertidal and deep sea floor and they are most abundantly present in the fine grained sediments to mild water current sandy beaches (Amaral *et al*., 2003 and McLachlan and Brown, 2006) [1, 27]. Marine polychaetes have gained recent interests for the isolation of novel and potential bioactive molecules from these unusual resources. These animals adapt special type of host defense mechanism which may differ from the immune system of the vertebrates (Banerjee *et al*., 2004) [3]. Some polychaetes have shown to produce hemagglutinins also known as 'lectins' generally are proteins or glycoproteins that specifically bind to the mono or oligosaccharides of non-immune origin with high affinity and specificity on a reversible manner (Van-Damme *et al*., 2003) [39]. These carbohydrate binding proteins mediate many biological functions including recognition of cell-cell or cell-matrix interactions by binding with various sugar groups ranged from polysaccharides to glycoproteins and glycolipids (Lis and Sharon, 1998) [24].

Naturally occurring protenious lectins cause toxicity to the agricultural pests can be used in bio-insecticides by spraying or via transgenic plants and studies have reported the toxic effects of several proteins when ingested by the insect pests (Van Damme, 2008) [38]. Vandenborre *et al*., 2009 [40] have reported the insecticidal proteins belonged to the class of lectins. These glycoproteins showing specific affinity to many mono or di-saccharides and they are widely been reported in bacteria, fungi, viruses, plants and animals (Peumans and Van Damme, 1995, Kilpatrick, 2002 and Singh *et al*., 2010) [32].

Mealybugs (Hemiptera: Pseudococcidae) are the plant sap-sucking, soft bodied, cottony appearance insects with more than 2000 species considered as the major pests of agriculture (Gullan and Kosztarab, 1997) [16]. They form white, waxy colonies on the leaves and stems, and suck the sap of the plants. The pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) was first described as *Phenacoccus hirsutus* Green on a Indian specimen collection on a shrum (Green, 1908) [15]. These native species south Asian spread the world and attacking more than 200 plants, including trees and shrubs and causing economic loss to various crops (Garland, 1998). In India, mealybugs infestations has been reported in different *Hibiscus species* (Raju *et al*., 1988), and in cotton (Muralidharan and Badaya, 2000) [20]. Hence, the present aimed to evaluate the insecticidal activity of lectin purified from a marine polychaete against the mealybugs infestation in *Hibiscus* plants.

Materials and methods

1. Collection of polychaete samples

Polychaetes samples were collected from the Gadilam estuary (N11 º44'11" and E79º47'12") in Cuddalore district, Tamil Nadu, India during the low tide as described by Masero *et al*., 1999. Collected polychaetes were washed with distilled water and stored at -20°C until further use.

Fig 1: Showing hibiscus mealybug insect

2. Identification of the polychaetes

Collected Polychaetes were identified up to species level based on their morphology, colour pattern, legs, head, mouth-parts arrangements and genetalia using the standard keys as described by Silva (1961), Day (1967), Fauchald (1977) [12], Uebelacker and Johnson (1984) [37].

3. Extraction of lectin from polychaetes

Extraction of lectin from polychaetes was done using the method described by Kawsar *et al*., 2009a [21]. About 200g by wet weight of frozen marine polychaetes (identified as *Perinereis cultrifera*) were dispersed into paste with a help of a razor blade and were homogenized with 10 volumes (w/v) of Tris buffered saline (TBS) pH 7.4, containing 0.9% NaCl along with 10mM of protease inhibitor mixture (Merck, India). The homogenate was filtered through a nylon filter and the filtrate was centrifuged at 14,720 g in 500 ml centrifuge bottles at 4˚C for 40 min. The supernatant was separated and centrifuged at 27,500 *g* at 4˚C for 30 min. The precipitate was collected and mixed again with 3 volumes (w/v) of TBS containing 10 mM EDTA and 50 mM galactose and this was incubated overnight at 4˚C. After incubation, the homogenate was centrifuged at 27,500 *g* for 40 min at 4˚C. The supernatant was collected and dialyzed against TBS to remove the galactose. The non-dialyzable fraction was recovered and used for further study.

4. Hemagglutinating activity

The hemagglutination activity of the extracted polychaete (*P. cultrifera*) lectin designated as "PcL" was done against rabbit erythrocytes as described by Matsui (1984) and Goto-Nance *et al.*, 1995. Hemagglutination assay was performed by adding 25µL of 2% (w/v) erythrocytes prepared in TBS containing 1% Triton X-100 (Merck, India) and 25µL of dialyzed, two fold-serially-diluted in TBS lectin (ranged from 1:1-1:1024) in 96 well V-shape titer plates and were incubated at room temperature for 1 h. The hemagglutination activity of the lectin was expressed as the titer (hemagglutination unit (HU), defined as the reciprocal of the highest dilution giving positive hemagglutination.

5. Lectin purification

Purification of lectin from the marine polychaete *P. cultrifera* extract was done using a series of steps such as varying concentration (20-80%) of ammonium sulphate saturation levels followed by desalting using dialysis against TBS pH 7.4 (partial purification). (Lowry *et al.*, 1951) [25]

6. Evaluation of insecticidal activity of purified lectin against *Hibiscus* mealybugs

Maintenance of the experimental animals

Fresh, healthy, uninfected young *Hibiscus* plant shoots along with the leaves and stems that were pre-rinsed with 2% bleach for 2 min. followed by a rinse with distilled water for 5 min. to remove the dirt on the plants and it was used as the diet throughout the study period in the control group. Fresh feeds that were treated in the above similar fashion were used for every 6 h to maintain the freshness of the feed. Twenty *Hibiscus* mealybug insects with equal number of both male and female sexes were taken in separate sterile Petri dishes. Animals in the Petri dishes covered with the lids and were maintained at 28°C±1°C with the relative humidity of 60-70% and 16:8 (light: dark) photoperiod for 24-72 h.

Preparation of the bio-insecticide and experimental design

Lectin purified from *P. cultrifera* was evaluated for its insecticidal activity potential against mealybugs infesting the *Hibiscus rosa-sinensis* L plants. Pre-cleaned plants sprayed with different concentrations (1.0-10.0 mg/ml at an interval of 1 mg/ml) of lectin purified from *P. cultrifera* prepared in Tris buffered saline (TBS) pH 7.4, containing 0.9% NaCl) was used for testing insecticidal activity (Febvaye *et al*., 1988, Douglas *et al*., 2006, Shahidi-Noghabi *et al*., 2008, and Sadeghi *et al*., 2009) [9, 13, 36]. Insect groups fed with pre-cleaned plants sprayed only the TBS served as the control. At the end of each day of the experimental period (i.e. 24-72 h), insects were observed and the percentage of mortality was calculated. The mortality rate and the half leathel concentration i.e., the lectin concentration kills 50% of the mealybug insects (LC_{50}) were calculated and expressed in percentage. The experiment was also conducted on the field with the above concentrations (1.0-10.0 mg/ml) of lectin mixed with 1% glycerol and sprayed over the mealybugs infested *Hibiscus* plant for a peroid of three days. The effect of lectin on mealybugs mortality were monitored visually.

Results and discussion

Polychaetes are the important organisms in the sandy beaches of tropical webs due to their abundance and variety of feeding types and they act as primary or secondary consumer (Checon *et al.*, 2017) [8]. Marine Polychaeta (Annelids) occupy intertidal areas to the deep sea and offering various bioactive compounds (Rodrigo and Costa, 2019). Lectins from the sea worms are only limited when compared to the other marine invertebrates. The first hemagglutinin, amphitritin, was isolated from the sea worm *A. ornate* (Garte and Rissell, 1976).

1. Collection of and identification of polychaetes

In the present study, polychaete samples were collected from the Gadilam estuary was identified as *Perinereis cultrifera* (Grube, 1840). Several early studies have reported the collection of *P. cultrifera* polychaetes in different parts of India (Sekar *et al.*, 2016, Bharathidasan *et al.*, 2017, Lazarus *et al.*, 2020 and Srilaxmi *et al.*, 2022) [5, 35].

2. Extraction of lectin from polychaetes

Lectins are found in plants, animals and microbes including viruses and as galactose is a key sugar participates in recognition, more than 60% of the lectins reported to date are mainly galactose specific lectins (Roopashri and Savitha, 2022). In the present study, lectin was extracted from *P. cultrifera* designated as 'PcL' was tested for hemagglutination activity. Dutta *et al.,* 2005 [10] isolated a galactose binding lectin from the serum of Indian catfish *C. batrachus*. Previous studies have reported lectin extraction from different animals Vasta *et al.,* 2011 [41] (from mammals) and Cammarata *et al.,* 2019 [7] from the mucus of a polychaete *S. spallanzani*.

3. Hemagglutination activity

The unique carbohydrate specificity property of lectins makes them as one of the most valuable tools in the purification and characterization of glycoproteins and also in the analysis of cell surface sugars. These lectins agglutinate RBCs, fibroblasts and lymphocytes (El-Araby *et al.,* 2020) [11]. In the present study, Hemagglutination activity of the curde lectin (PcL) was tested using different dilutions of PcL (ranged from 1:1-1:1024) against rabbit erythrocytes. The results showed a titer (as hemagglutination unit (HU) of 32 HU against rabbit erythrocytes. The results are in agreement with Hatakeyama *et al.,* 1994 [18], Goto-Nance *et al.,* 1995 who observed hemagglutination activity of lectin against rabbit erythrocytes and Kawsar *et al.,* 2009a [21], 2009b [20] and 2010 [22] reported in *P. nuntia* ver. vallata (Polychaeta), in a Japanese sea hare *A. kurodai* egg and *P. nuntia* lectins in against rabbit erythrocytes.

4. Purification of lectin

The crude polycheate extract added with 60% ammonium sulphate saturation gave the highest protein precipitation and hemagglutination activity. The precipitate was collected and dialysed against TBS pH 7.4. This partially purified dialysate was tested for hemagglutination activity and estimation of total protein and was further purified by using column chromatography. Early studies have reported different levels of ammonium sulphate concentration from different sources. For example, Bhagyashree *et al.,* 2011 [4] from *X. campestris* NCIM 5028 and Kamei *et al.,* 2023 [19] from the seeds of *M. buteiformis* Voigt. In the present study, the total protein content in the partially purified lectin (PcL) sample showed a total protein content of 22 mg/ml. Desmiaty *et al.,* 2024 reported a total protein concentration range 1284.83-2947.33µg/ml in a *C. esculenta* L. rhizome lectin.

Table 1: Purification steps of lectin isolated from the marine polychaete *P. cultrifera*

Purification steps	Vol. (ml)	Total Protein (mg)	HA titer (HU)	THA (in HU)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude cell free extract	100	22.7	32	3200	141	1	100
Ammonium sulphate precipitation and dialysis	18	6.2	128	2304	372	2.6	72
DEAE-Cellulose column	8	2.3	256	2048	890.4	6.3	64
DEAE-Sephadex A-50 column	6	1.2	256	1536	1280	9	48

HA: Hemagglutination activity, **HU:** hemagglutination unit, **THA:** Total hemagglutination activity, Vol.: Volume of sample extract

5. Insecticidal activity of purified lectin against *Hibisucs* mealybugs

Though, several plant based lectins and microbial lectins are shown to have insecticidal activities, there are no or only very few studies have reported lectins from animal origin with insecticidal activity. Thus, the present study was aimed to evaluate the insecticidal activity of a lectin purified from a marine polychaete *Perinereis cultrifera* against *Hibiscus* mealybugs causing infection in *Hibiscus* plant.

In the present study, different concentrations (1-10 mg/ml at an interval of 1 mg/ml) of lectin purified from *P. cultrifera* were used for testing the insecticidal activity against *Hibiscus* mealybugs. The results showed excellent insecticidal activity on a dose dependent manner with the highest insect mortality of 73.33% at 10 mg/ml of lectin concentration and the lowest mortality rate of 8.33% at 1 mg/ml concentration. The lectin concentration required to kill 50% of the mealybug insects (LC_{50}) was found to be 6.8 mg/ml (Table 2). The field study showed effective insecticidal activity after 3 days of application.

The results of the present study is supported by the report of Sadanandan and Rauf (2021) who observed a dose dependent *A.craccivora* mortality ranged from 13.33 % to 100% for a marine sponge *F. cavernosa* lectin (FCL) against cowpea aphid, *A. craccivora* by artificial diet. The present study also showed a dose dependent mortality against mealybug insects. To the best of our knowledge, this is the first report on the insecticidal activity of a marine polychaete lectin isolated from *P. cultrifera* against mealybugs infestation in *Hibiscus* plants.

Hamshou *et al*., 2010 [17] observed a median insect toxicity for a *S. sclerotiorum* agglutinin (SSA) with a LC_{50} of 66mg/ml against pea aphid (*A. pisum*) fed with an artificial diet containing different concentrations of lectin. Khoobdel *et al*., 2022 [23] used lectin extracted from *P. persicaria* L. as a bio-control agent against rice weevil *S. oryzae* L. they fed the insects with carob extract diet as a food mixed with the lectin and observed a LC_{50} value of 3.68%.

Lectins isolated from the legumes are toxic to a broad spectrum of insects representing several orders including, Coleoptera, Diptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera and Neuroptera. For example, Coelho *et al*., 2007 reported in *C. cephalonica* (Lepidoptera), Macedo *et al*., 2007 and Oliveira *et al*., 2011 [30] against *C. maculatus* (Coleoptera), and *Z. subfasciatus* (Coleoptera) respectively. Lectin isolated from *R. solani* and *S. nigra* showed permeability of lectins through the above mentioned membranes of a red flour beetle *T. castaneum* (Dandagi *et al*., 2006). Also, the size, charge of of the perritrophic membrane pores and molecular dimensions determined the membrane permeability of lectins (Walski *et al*., 2014) [42]. Schachter, 2009 [34] reported entomotoxic activity of agglutinin (GNA) from a snowdrop plant (*G. nivalis*) which has specific binding affinity to the terminal mannose residue of the high mannose containing N-glycans that are found to be rich on the insect glycoproteins. The GNA lectin also was found to be effective against sucking insects.

The mode of action of insecticidal lectins might be due to their binding to a chitin rich peritrophic membrane and the peritrophic gel or the microvilli of epithelial cells in the midgut of the insects and this cause a lectin induced retardation of insects' bio-physiological functions and their mortality. Lectins also cause toxicity in insects with the increased detoxifying and antioxidant enzymes (Macedo *et al*., 2003, Zhu-Salzman and Salzman, 2001 [44] and Rahimi *et al*., 2018) [33]. Lectins could interfere with the digestion and absorption nutrients by the inhibition of digestive enzymes and proteins responsible for assimilation (Zhu *et al*., 1996) [43] and they are resistant to the gut proteases of the insects (Macedo *et al*., 2002) [26].

Lectins cause insecticidal activity by directly binding to the glycoconjugates of the cell epithelium of the insects that lack the peritrophic membrane (Roy *et al*., 2014). Lectins cause insecticidal activity by binding on the surface of the epithelial cells that are rich glycoproteins and this binding may differ based on the various sugars present on the insects with different physiological functions. It is opinied that clathrin-mediated endocytosis may be the underlying mechanism in the permeability of lectins through the midgut epithelial cells. However, the exact mechanism is not yet fully understood (Francis *et al*., 2003 and Caccia *et al*., 2012) [6, 14].

Table 2: Insecticidal activity of purified lectin against *Hibiscus* mealybugs

Purified lectin concentration (mg/ml)	Mortality rate (%)
Control	- ND -
1.0	8.33
2.0	10
3.0	16.66
4.0	26.66
5.0	28.3
6.0	43.33
7.0	51.66
8.0	58.33
9.0	66.66
10.0	73.33

ND: Not detected

Conclusion

The present study on evaluation of insecticidal activity of lectin purified from a marine polychaete collected from the Gadilam estuary in Cuddalore district against the mealybugs infestation in *Hibiscus* plants showed potential insecticidal activity and could be used for the bio-control of mealybugs infestations in *Hibiscus* plants.

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References

1. Amaral ACZ, Denadai MR, Turra A, Rizzo AE. Intertidal macrofauna in Brazilian subtropical tide-dominated sandy beaches. J Coast Res,2003:SI35:446-55.
2. Andrea WT, Jose RC, Edilma S, Edgar R, Jose FA, Annabelle V, *et al*. Purification and biochemical characterization of a T/TN specific lectin from *Lepechinia bullata* seeds (Lamiaceae). Int J Pharm Pharm Sci,2017:9(11):165-74.
3. Banerjee S, Chaki S, Bhowaland J, Chatterjee BP. Mucin binding mitogenic lectin from freshwater Indian gastropod *Belamyia bengalensis*: Purification and molecular characterization. Arch Biochem Biophys,2004:421:125-34.
4. Bhagyashree J, Jayant K, Hephzibah S, Maksudul K. Purification and characterization of a lectin from *Xanthomonas campestris* NCIM 5028. Can J Microbiol,2011:42:609-12.
5. Bharathidasan V, Partha Sarathy P, Selvaraj P, Murugesan P. Sexual reproduction and larval development of nereididae polychaeta *Perinereis cultrifera* (Grube, 1840). Int J Sci Invent Today,2017:6(6):772-82.
6. Caccia S, Van Damme EJ, De Vos WH, Smagghe G. Mechanism of entomotoxicity of the plant lectin from *Hippeastrum hybrid* (amaryllis) in *Spodoptera littoralis* larvae. J Insect Physiol,2012:58:1177-83.
7. Cammarata M, Benenati G, Dara M, Parisi MG, Piazzese D, Falco F, *et al*. *Sabella spallanzanii* mucus contains a galactose-binding lectin able to agglutinate bacteria. Purification and characterization. Invertebr Surviv J,2019:16(1):15-24.
8. Checon HH, Pardo EV, Amaral ACZ. Breadth and composition of polychaete diets and the importance of diatoms to species and trophic guilds. Helgol Mar Res,2017:70:1-19.
9. Douglas AE, Price DRG, Minto LB, Jones E, Pescod KV, Francois CLMJ, *et al*. Sweet problems: insect traits defining the limits to dietary sugar utilisation by the pea aphid, *Acyrthosiphon pisum*. J Exp Biol,2006:209:1395-403.
10. Dutta S, Sinha B, Bhattacharya B, Chatterjee B, Mazumder S. Characterization of a galactose binding serum lectin from the Indian catfish, *Clarias batrachus*: Possible involvement of fish lectins in differential recognition of pathogens. Comp Biochem Physiol,2005:141:76-84.
11. El Araby MM, El Shatoury EH, Soliman MM, Shaaban HF. Characterization and antimicrobial activity of lectins purified from three Egyptian leguminous seeds. AMB Expr,2020:10(1):1-14.
12. Fauchald K. The polychaete worms: Definitions and keys to the orders, families, and genera. Nat Hist Mus LA County Sci Ser,1977:28:1-190.
13. Febvaye G, Delobel B, Rahbé Y. Influence of the amino acid balance on the improvement of an artificial diet for a biotype of *Acyrthosiphon pisum* (Homoptera: Aphididae). Can J Zool,1988:66:2449-53.
14. Francis F, Marty-Detraves C, Poincloux R, Baricault L, Fournier D, Paquereau L. Fungal lectin, xcl, is internalized via clathrin-dependent endocytosis and facilitates uptake of other molecules. Eur J Cell Biol,2003:82:515-22.
15. Green EE. Remarks on Indian scale insects (Coccidae), Part III. With a catalogue of all species hitherto recorded from the Indian continent. Mem Dep Agric India Ent Ser,1908:2:15-46.
16. Gullan PJ, Kosztarab M. Adaptations in scale insects. Annu Rev Entomol,1997:42:23-50.
17. Hamshou M, Smagghe G, Shahidi-Noghabi S, Geyter ED, Lannoo N, Van Damme EJM. Insecticidal properties of *Sclerotinia sclerotiorum* agglutinin and its interaction with insect tissues and cells. Insect Biochem Mol Biol,2010:40:883-90.

18. Hatakeyama T, Kohzaki H, Nagatomo H, Yamasaki N. Purification and characterization of four Ca2+-dependent lectins from the marine invertebrate *Cucumariae chinata*. J Biochem,1994:116:209-14.
19. Kamei R, Devi OS, Singh SJ, Singh WS, Singh SS. Purification and characterization of a novel D-galactose binding lectin from seeds of *Meizotropis buteiformis*. Curr Pharm Biotechnol,2023:24(5):665-75.
20. Kawsar SM, Matsumoto R, Fujii Y, Yasumitsu H, Dogasaki C, Hosono M, *et al*. Purification and biochemical characterization of a D-galactose binding lectin from Japanese sea hare (*Aplysia kurodai*) eggs. Biochem,2009b:74(7):709-16.
21. Kawsar SMA, Takeuchi T, Kasai KI, Fujii Y, Matsumoto R, Yasumitsu H, *et al*. Glycan-binding profile of a D-galactose binding lectin purified from the annelid, *Perinereis nuntia* var. *Vallata*. Comp Biochem Physiol B,2009a:152:382-9.
22. Kawsar SMA, Sheikh U, Hidetaro Y, Yasuhiro O. The cytotoxic activity of two D-galactose-binding lectins purified from marine invertebrates. Arch Biol Sci,2010:62(4):1027-34.
23. Khoobdel M, Rahimi V, Ebadollahi A, Krutmuang P. Evaluation of the potential of a lectin extracted from *Polygonum persicaria* L. as a biorational agent against *Sitophilus oryzae* L. Molecules,2022:27:793. Available from: https://doi.org/10.3390/molecules27030793
24. Lis H, Sharon N. Lectins: Carbohydrate-specific proteins that mediate cellular recognition. Chem Rev,1998:98:637-74.
25. Lowry OH, Rosebrough NJ, Farr AL. Protein measurement with the Folin phenol reagent. J Biol Chem,1951:193:265-75.
26. Macedo MLR, Freire MGM, Novello JC, Marangoni S. *Talisia esculenta* lectin and larval development of *Callosobruchus maculatus* and *Zabrotes subfasciatus* (Coleoptera: Bruchidae). Biochim Biophys Acta,2002:1571:83-8.
27. McLachlan A, Brown AC. The ecology of sandy beaches. 2nd ed. Massachusetts: Academic Press, 2006.
28. Milind V, Vikram S, Vijay U, Rajeshwar S. Extraction and purification of lectin from soybean seeds (*Glycine max*). Lett Appl NanoBioSci,2023:12:1-9.
29. Muralidharan CM, Badaya SN. Mealy bug (*Maconellicoccus hirsutus*) outbreak on herbaceum cotton (*Gossypium herbaceum*) in Wagad cotton belt of Kachch. Indian J Agric Sci,2000:70:705-6.
30. Oliveira CFR, Luz LA, Paiva PMG, Coelho LCBB, Marangoni S, Macedo MLR. Evaluation of seed coagulant *Moringa oleifera* lectin (cMoL) as a bioinsecticidal tool with potential for the control of insects. Process Biochem,2011:46:498-504.
31. Oliveira MDL, Andrade CAS, Santos-Magalhães NS, Coelho LCBB, Teixeira JA, Cunha MGCD, *et al*. Purification of a lectin from *Eugenia uniflora* L. seeds and its potential antibacterial activity. Lett Appl Microbiol,2008:46:371-6.
32. Peumans WJ, Van Damme EJM. Lectins as plant defense proteins. Plant Physiol,1995:109:347-52.
33. Rahimi V, Hajizadeh J, Zibaee A, Jalali Sendi J. Effect of *Polygonum persicaria* (Polygonales: Polygonaceae) extracted agglutinin on life table and antioxidant responses in *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae. J Econ Entomol,2018:111:662-71.
34. Schachter H. Paucimannose N-glycans in *Caenorhabditis elegans* and *Drosophila melanogaster*. Carbohydr Res,2009:344:1391-6.
35. Sekar V, Rajasekaran R, Sachithanandam V, Sankar R, Sridhar R, Kingsley PW. Species diversity of polychaete in coral reef ecosystem of Great Nicobar Island, India. Nusantara Biosci,2016:8(1):71-6.
36. Shahidi-Noghabi S, Van Damme EJM, Smagghe G. Carbohydrate-binding activity of the type-2 ribosome-inactivating protein SNA-I from elderberry (*Sambucus nigra*) is a determining factor for its insecticidal activity. Phytochemistry,2008:69:2972-8.
37. Uebelacker JM, Johnson PG. Taxonomic guide to the polychaetes of the Northern Gulf of Mexico. Vol. 5. Barry A. Vittor and Associates, 1984, 32-34.
38. Van Damme EJM. Plant lectins as part of the plant defence system against insects. In: Schaller A, editor. Induced plant resistance to herbivory. Dordrecht: Springer, 2008, 285-307.
39. Van-Damme EJM, Lannoo N, Fouquaert E, Peumans WJ. The identification of inducible cytoplasmic/nuclear carbohydrate binding proteins urges to develop novel concepts about the role of plant lectins. Glycoconj J,2003:20(7-8):47.
40. Vandenborre G, Van Damme EJM, Smagghe G. Natural products: plant lectins as important tools in controlling pest insects. In: Ishaaya I, Horowitz AR, editors. Biorational control of arthropod pests: application and resistance managements. Dordrecht: Springer-Verlag, 2009, 163-87.
41. Vasta GR, Nita-Lazar M, Giomarelli B, Ahmed H, Du S, Cammarata M. Structural and functional diversity of the lectin repertoire in fish: relevance to innate and adaptive immunity. Dev Comp Immunol,2011:35:1388-99.
42. Walski T, Van Damme EJM, Smagghe G. Penetration through the peritrophic matrix is a key to lectin toxicity against *Tribolium castaneum*. J Insect Physiol,2014;70:94-101.
43. Zhu KRA, Bressan RA, Hasegawa PM, Murdock LL. Identification of N-acetylglucosamine binding residues in *Griffonia simplicifolia* lectin II. FEBS Lett,1996:390:271-4.
44. Zhu-Salzman K, Salzman RA. Functional mechanics of the plant defensive *Griffonia simplicifolia* lectin II: Resistance to proteolysis is independent of glycoconjugate binding in the insect gut. J Econ Entomol,2001:94:1280-4.