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Bio-efficacy of pumpkin phloem lectin on red spider mite, *Oligonychus coffeae* (Nietner) infesting tea

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Red spider mite (RSM) (*Oligonychus coffeae* Nietner), (Acarina: Tetranychidae) infestation is a limiting factor in tea productivity. Severe infestation leads to defoliation of maintenance leaves, which ultimately affects the bush physiology and bush health. Control measures of RSM are mainly achieved by the use of synthetic acaricides (Amarasena *et al.*, 2011). Emphasis is given for the application of eco-friendly botanical pesticides for the pest management in tea. Number of botanical pesticides was reported to control several insect pests (Prakash and Rao, 1997; Naveena *et al*., 2010). Bio-efficacy of plant lectin for insect control has already been reported (Czapla and Lang 1990; Roy *et al*., 2008). Pest resistant crops attract attention in a world which is dominated by the use of chemical insecticides. Lectins are carbohydrate binding proteins present in a range of organisms which are well characterized in plants and posses defense properties like anti-fungal, anti-bacterial and insecticidal (Lis and Sharon, 1998; Peumans and Van Damme, 1995). Bio-efficacy of garlic bulb lectin on *O. coffeae* infesting tea has been proved by Roy *et al*. (2008). The present study was aimed at evaluating the bio-efficacy of chito-oligosaccharide/chitin binding lectin of *Cucurbita maxima* (pumpkin) on RSM in the laboratory.

Pumpkin fruit obtained from the local market was peeled off, deseeded and the edible part was used in crude lectin isolation. Purification of pumpkin phloem lectin (PPL) was accomplished with affinity chromatography on chitin column following Narahari and Swamy (2010). Mites were collected from the infested tea fields of UPASI Experimental Farm, Valparai, Tamil Nadu, India. The mites were maintained and mass cultured in laboratory using the method described by Roobakkumar *et al*. (2010). Two laboratory bio-assays were conducted with purified protein on RSM mortality and ovipositional deterrence individually. Each treatment was replicated five times. Matured tea leaves without any RSM infestation were collected from the field and leaf discs of 2 cm diameter were placed on moist cotton in a Petri dish (9 cm diameter). Different concentrations of PPL (10, 20, 30, 40 and 50 µg ml^{-1}) was overlaid on the leaf discs and allowed to dry at room temperature. Leaf discs overlaid with distilled water served as control. Ten adult RSM (less than 48 h old) were transferred from the stock onto the PPL treated leaf discs. Plates were maintained in the room temperature and the treated leaf discs were observed after 24, 48, 72 and 96 hours under binocular microscope (Olympus No.1220). Ovipositional deterrence was studied by allowing the 10 adult females (individuals/treatment) to lay eggs both on the PPL treated and untreated control leaf discs. The degree of deterrence was assessed in terms of difference in the number of eggs deposited by the females on control and treated leaves.

For lectin purification pumpkin fruit (100 g) was homogenized with 100 ml of 0.1 M PBS-β-ME (phosphate buffer saline containing β- mercapto-ethanol) (1:1 v/w) contains 20 mM sodium phosphate buffer, pH 7.4, 150 mM NaCl, 0.02 per cent sodium azide and 10 mM β - mercaptoethanol. Purified PPL was estimated using Bradford method (1976) and about 10 µg of protein was analyzed on 15 per cent SDS-PAGE for its subunit molecular weight in accordance with Laemmli (1970). Native

PAGE was run without adding SDS in gel as well as in running buffer. Haemagglutination and its inhibition assays were carried out in 96 well micro titer plates using 4 per cent rabbit erythrocytes described by Narahari and Swamy (2010) and the agglutination was observed visually. Data generated from bioassay were subjected to statistical analysis (ANOVA) and results presented in accordance with Duncan's multiple range test (DMRT). The Discrimination Quotient (DQ) was calculated using the formula, DQ = C-T/C+T, where C = number of eggs on control leaves; T = number of eggs on treated leaves.

Whole pumpkin fruit was used for the isolation of crude protein, since the lectin in phloem part has the tendency to get bound to the column. All active fractions were lyophilized and used for further studies. In native PAGE (Fig. 1), lectin was resolved as a single band which confirmed the homogeneity of purified lectin (free from other proteins of pumpkin phloem). Pumpkin lectins were reported to be existing as a dimer (Bostwick *et al*., 1994). Presence of ~23 kDa single band in SDS-PAGE (Fig. 2) indicating that subunits of the PPL are having the same molecular weight homodimer (Narahari and Swamy, 2010). Lectin from snake gourd (Cucurbitaceae) was reported as a hetero-dimer by Narahari and Swamy (2011) which demonstrated the diversity of lectin structure within the family. Biological and bio-ecological studies on RSM have already been reported (Vitarana, 2000; Babu *et al*., 2010). In the present study, PPL bioassay against RSM resulted in total mortality in 96 h at 50 µg ml^{-1} concentration (Table 1). Survival of individual mites was observed by touching each mite with a single hair brush. Mites that were unable to move were considered dead and those which were

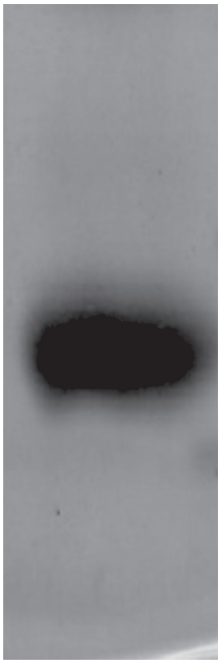

Fig. 1. Native PAGE analysis of PPL
Note: Presence of single band indicates the homogeneity of lectin preparation

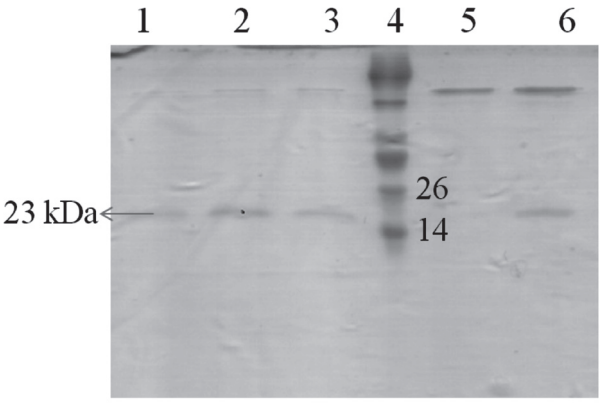

Fig. 2. SDS-PAGE analysis of PPL
Lane 1, 2, 3- purified PPL (~23kDa), Lane 4- protein marker, Lane 5, 6- unbound pumpkin phloem proteins of chitin column

Table 1. Influence of PPL on RSM mortality with reference to incubation time

PPL (µg ml^{-1})	Mortality (%) after			
	24 h	48 h	72 h	96 h
Control	00 ± 00 (1.00)a	00 ± 00 (1.00)a	00 ± 00 (1.00)a	00 ± 00 (1.00)a
10	24 ± 2.1(5.00)b	34 ± 2.4 (5.92)b	46 ± 2.4 (6.86)b	52 ± 2 (7.28)b
20	36 ± 2.4(6.08)c	50 ± 3.1 (7.14)c	54 ± 2.4 (7.42)c	60 ± 3.1 (7.81)c
30	44 ± 2.4(6.71)d	56 ± 2.4(7.55)cd	60 ± 3.1 (7.81)c	70 ± 3.1 (8.43)d
40	54 ± 2.4(7.42)e	62 ± 2.0 (7.94)d	72 ± 3.7 (8.54)d	82 ± 3.7 (9.11)e
50	66 ± 2.4(8.19)f	74 ± 2.4 (8.66)e	82 ± 2.0 (9.11)e	100 ± 0 (10.04)f

Note: Figures denote mean of five replicates followed by ± standard error. Figures in the parenthesis are transformed values of $(x+1)^{0.5}$. Figures followed by the same alphabet in a column do not differ significantly at P = 0.05 according to DMRT

alive were counted. Slow movement of RSM was noticed after 24 h in test plates when compared to the control. Dose and time dependant mortality was observed in the present study. As the concentration of lectin increased, mortality rate of RSM enhanced concurrently (Table 1). Incubation period also played an important role on RSM mortality where ~60 per cent mortality was observed even at lower concentration. Results of this study showed the potential of PPL as insecticidal by causing significant mortality on RSM. In a similar kind of study, this statement was accordance with Roobakkumar *et al*. (2010) where he tested the garlic aqueous extract, neem kernel aqueous extract and pongam kernel aqueous extract which were effectively used for the control of RSM.

Lectins are different in their specificity; for instance, Roy *et al*. (2008) reported the toxicity of mannose binding garlic bulb lectin against RSM. Specificity of the garlic bulb lectin and pumpkin phloem lectin is different because the former is mannose specific and the latter is specific to chito-oligosaccharides. Mannose specific lectin target site in the insect is glycoproteins present in brush border membrane vesicles while chitin binding lectins have target site in the glycoproteins of peritrophic membrane which contains chitin like structures (Macedo *et al*., 2008). Huesing *et al*. (1991) have demonstrated the toxicity of chitin binding wheat germ agglutinin (WGA), rice lectins and nettle lectins against *Callosobruchus maculates.* It has been assumed that PPL may bind to the chitin or chitinacious structures (digestive enzymes or the proteins) present inside the body of RSM. However, further studies are required in this line. Since RSM has a short life span, the population density attains its peak in short duration which leads to severe crop loss. Hence, control of their population demands immediate attention. Ovipositional deterrence which is considered as significant characteristic feature in RSM control and discrimination quotient value of different concentrations of PPL on RSM is given in Table 2. DQ ranging from 0 to 1 is an index for determination of the effect of PPL on RSM's ovipositional behaviour. DQ value of 0.52 was achieved at 50 µg ml^{-1} of PPL which means that the adult mites showed discrimination among the treated leaves with respect to number of eggs laid. RSM laid lesser number of eggs on PPL treated leaves than the untreated control. After 96 h 100 per cent mortality was achieved hence the eggs were laid before 96 h indicating the moderate interference of lectin in its ovipositional behavior. In a previous study, seed and leaf extracts of *Datura stramonium* L. decreased the oviposition in two-spotted red spider mite, *T. urticae* (Koch) (Kumral *et al.,* 2010). Application of acetone extract of garlic bulb on leaves of *Phaseolus vulgaris* reduced fecundity of *T. urticae* (Barakat *et al.,* 1986). Roobakkumar *et al*. (2010) has reported ovipositionl deterrence by garlic bulb aqueous extract and neem kernel aqueous extract when sprayed on RSM. The need of the hour is to identify an alternative method for chemical control of pest and an eco-friendly way of pest management. In addition, the fact that a lectin present in an edible plant may contribute towards an easier acceptance as an insect resistance factor in transgenic crop plants (Sadeghi *et al.,* 2008). From the results, PPL may be considered to be a potent control component of integrated pest management (IPM) due its propensity to control the survival rate and fecundity of the RSM and its usefulness in affecting the normal metabolism of RSM. Detailed study of the mechanism of lectin action in mite will strengthen its application in biotechnology.

Table 2. Ovipositional deterrence and DQ value of PPL on RSM

PPL Dose (µg ml^{-1})	No. of eggs observed at 96 h*	DQ value
10	85.6 ± 6.94 (9.31)e	0.12
20	73.8 ± 2.15 (8.65)d	0.19
30	63.8 ± 2.80 (8.05)c	0.26
40	43.2 ± 2.17 (6.65)b	0.43
50	34.0 ± 1.64 (5.92)a	0.52
Control	109.4 ± 4.41 (10.51)f	0.00

Note: *Values represent mean of five replications ± SE. Values in the parenthesis are transformed values of (x+1) 0.5. Figures followed by the same alphabet in a column do not differ significantly at P = 0.05 according to DMRT

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