Pest control potential of *Cyclosorous interruptus, Christella dentata* and *Nephrolepis cordifolia* on the biology of *Spodoptera litura* (Fab.)

Selvaraj, P. Princy Rathnamala Jayaseeli, J. and Mary Ansilin.

ABSTRACT

The present experiment was designed to investigate the pesticidal property of the chosen ferns *viz., Cyclosorus interruptus* (Willd.) H.Itô (Theylepteridaceae), *Christella dentata* (Willd.) (Forssk.) Brownsey et Jermy (Theylepteridaceae), and *Nephrolepis cordifolia* (L.) Presl. Phytochemical analysis carried out using methanol extracts of the experimental ferns revealed the presence of the secondary metabolites like alkaloids, steroids, tannins, flavonoids, cardiac glycosides and phenolic compounds in methanolic extracts of the experimental ferns. The nano particles of chosen fern extracts synthesized using silver nitrate ($AgNO_3$) showed the characteristic colour change from pale yellow to dark brown indicated the synthesis of silver based nano particles. The UV- Visible spectrum obtained for the silver nanoparticles (AgNPs) of the experimental ferns confirmed the synthesis of nanoparticles (433.50 nm, 447.00 nm and 444.00 nm for *C. interruptus, C. dentata* and *N. cordifolia*, respectively). Bioassay on the pesticidal property of the methanol extracts and ferns-AgNP were evaluated against third instar larvae of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). The results of the bioassay revealed the impact of methanolic extracts and their AgNP particles on the developmental period, pupal weight, percentage of pupation and adult emergence and also caused larval, pupal and adult deformities that confirm the insecticidal activity of the experimental ferns.

Keywords: *Cyclosorous interruptus, Christella dentata, Nephrolepis cordifolia, Spodoptera litura,* nano particles, pesticidal property.

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INTRODUCTION

Insect herbivoury accounts for a loss of 15% crop yield annually. Among them polyphagous Lepidopterans are the most notorious pests infesting a number of agricultural crops (David, 1992, Swaminathan, 1998, *Spodoptera litura* (Lepidoptera: Noctuidae) is a polyphagous and cosmopolitan lepidopteran insect pest infests more than 140 species of economically important agriculture pests globally, including southeast Asia, India, China, and Japan (Wheeler and Isman, 2001; Zhang *et al.*, 2012; Xu *et al.*, 2015). Wide arrays of plants are being screened as an alternative to the synthetic chemical pesticides. This global hunt reported a number ecofriendly, biologically safer molecules with potential pesticidal and antimicrobial activity (Muraleedharan and Sheeladevi, 1992). Among pteridophytes, *Cheilanthes tenuifolia* (Faux *et al.*, 1970), *Polypodium vulgare, Pteridium aquilinum, Seratula tinctora* and *Cheilanthes farinosa* (Rajkumar *et al.*, 2000) *Christella parasitica, Pteridium aquilinum* and *Hemionitis aurifolia* (Selvaraj, 2002) were reported to contain insecticidal properties. Hence the present experiment was designed to evaluate the pesticidal property of methanolic extracts and the silver based nano particles of *C. denta, C. interruptus* and *N. cordifolia* against *S. litura.*

MATERIALS AND METHODS

Collection of ferns and phytochemical analysis

The experimental ferns *viz., C. dentata* and *C. interruptus* were collected from Kandervillagam, Colachel (8.178620 °N and 77.256096 °N) and *N. cordifolia* was collected from Athur, Thiruvattar (8.3348 °N, 77.2664°E), Kanyakumari District, Tamil Nadu, India. The ferns were washed thoroughly with tap water to remove debris and were shade dried in room temperature for two weeks. The dried ferns were partially ground and stored for further use. From this stock 100 gm of powder each were used separately for extraction using Soxhlet apparatus with methanol as solvent between 40 to 60 °C. The extracts were concentrated and stored for further use. From these stocks one percent solutions were prepared and were used for preliminary phytochemical analysis using standard procedure.

Preparation and UV-visible detection of AgNPs

Silver nitrate stock solution was prepared by dissolving 17 mg of silver nitrate (10^{-9} M $AgNO_3$) in 100 mL of distilled water in a conical flask and the pH is adjusted to 7. To induce the synthesis of silver nano particles, six different concentrations of plant extracts (25 µL, 50 µL, 75 µL, 100 µL, 125 µL and 150 µL) were added separately into 5 mL of $AgNO_3$ solutions taken in six different test tubes. The appearance of brown colour was considered as an indication of the synthesis of AgNPs. The best concentration in which the brown colour was observed was chosen for UV-vis Spectroscopic analysis between 200–500 nm (Shimadzu 1800 UV-Vis Spectrometer).

Pest collection and rearing

A laboratory stock culture of *S. litura* was established using field collections of adult males and females from groundnut fields around Tirunelveli. The adults were introduced into the ovipositor chamber with fresh castor leaves and sugar solution for egg laying. The adult female laid creamy white eggs covered with scales. The laid eggs were collected carefully in a Petridish using camel hair brush and were maintained under laboratory condition (28 ± 2 °C, RH 85% and Light period 14 L:10 D). The young larvae hatched after three days of incubation were fed with fresh tender castor (*Ricinus communis*) leaves and were reared under laboratory conditions.

Bioassay

Five grams of Castor leaves were soaked in four different concentrations (wt/v) (*viz.*, 0.5%, 1.0%, 1.5% and 2.0%) of methanolic extract and silver nano particles of *C. interruptus*, *C. dentata* and *N. cordifolia* separately for five minutes. For the control, leaves were soaked in methanol solvent. After five minutes, the leaves were air dried for another 10 min and were used for the bioassay. The experimental *S. litura* larvae (five each) were taken in plastic containers of 500 mL capacity and were provided with respective concentrations of experimental extract soaked caster leaves and the containers were covered with muslin cloth. Three replicates each were maintained for each experimental concentration and control respectively. Freshly treated leaves were provided to the experimental animals after cleaning and recording the observations on daily basis for four consecutive days. After the stipulated period of the experimental exposure (4 days) the animals were fed with normal diet (untreated castor leaves). The pupated insects were collected, cleaned and placed on moist cotton swaps in petridishes and kept in the adult emergence cages for further observation until adult emergence. The pupae were examined daily to note mortality or developmental abnormalities if any. This was continued up to the adult emergence or death of all the experimental animals. The adult moths emerged were collected and released into the oviposition chamber for oviposition.

Statistical analysis

Statistical analysis and graphical representation of the experimental data was carried out statistically using Microsoft Excel and SPSS 16.6.

RESULTS AND DISCUSSION

Phytochemical analysis

In the present study primary phytochemical analysis was carried out to identify the secondary metabolites responsible for insecticidal property of the experimental ferns *viz.*, *Cyclosorous interruptus, Christella dentata* and *Nephrolepis cordifolia*. The results of the analysis revealed the presence of alkaloids, steroids, tannins, flavonoids, cardiac glycosides and phenolic compounds in methanolic extracts (Table 1).

Table 1. Phytochemical analysis on methanol extract of the experimental ferns, *C. dentata, C. interruptus* and *N. cordifolia*

Secondary metabolites	***C. dentata***	***C. interruptus***	***N. cordifolia***
Alkaloids	+	+	–
Steroids	+	+	–
Tannins	+	+	+
Saponoids	–	–	–
Flavonoids	+	+	+
Terpenoids	+	+	+
Cardiac glycosides	+	+	–
Phenolic compounds	+	+	+
Aromatic acids	–	–	–
Xanthoproteins	–	–	–

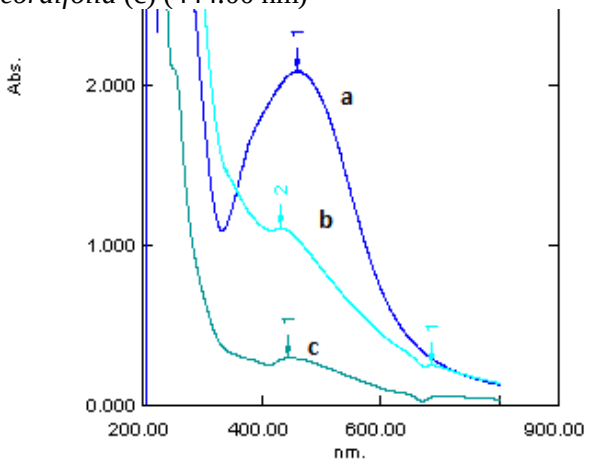
+Indicates present and – indicates absent

Britto and Manickam (1992), Jesudass (1997) and Selvaraj (2002) also reported the occurrence of phytochemical constituents such as tannins, phenolics, flavonoids, terpenoids, and saponin in their experimental pteridophytes. Presence of steroids, tannins, lignins, flavonoids and phenolic compounds were also reported by Tanaka *et al.* (1981); Kamaya *et al.* (1996) and Jesudas (1997). Generally plants are reported to accumulate massive quantities of secondary metabolites to defend phytophagy. Of these tannins and steroids are known for their insecticidal properties (Sundararajan and Kumuthakalavalli, 2000; Ananthakrishnan, 2002; Selvaraj, 2002).

Synthesis and UV-Visible spectrophotometric analysis of nano particless of ferns

In the trials carried out for the synthesis of silver based nano particles the all the experimental extracts showed the characteristic colour change (formation of brown colour). When comparing the six different experimental concentrations of methanolic extracts of the experimental ferns, 150 µL concentrations showed the colour change to dark brown within two hours of preparation. Hence the 150 µL concentration samples were used for the UV-Vis spectrophotometric analysis between 200-500 nm. The spectrum obtained for the analysis showed a characteristic peak at around 430-450 nm. Among the three ferns tested *C. interruptus* recorded the maximum absorbance (1.109) at 433.50 nm followed by *C. dentata* 1.053 at 447 nm whereas *N. cordifolia* recorded the lowest absorbance (0.296) at 444 nm (Fig. 1).

Fig. 1. UV- Visible spectrum of AgNPs of *Christella dentata* (a) (450.00nm), *Cyclosorous interruptus* (b) (433.50nm) and *Nephrolepis cordifolia* (c) (444.00 nm)



Impact of fern extracts on *S. litura* larva

Irrespective of the experimental ferns, the methanolic extracts recorded a concentration dependent mortality during the experimental period (Table 2). Similar findings were also reported by Sahayaraj and Mary Jeeva (2012) and Ventrella *et al.* (2016). Among the experimental concentrations of methanolic extract, the highest mortality was observed in the highest concentration of *N. cordifolia* at 96-hrs (LC_{50}= 0.9177%), followed by *C. interruptus* (LC_{50}= 0.890%) and *C. dentata* (LC_{50}=0.765%). However, the Fern-AgNPs particles recorded very low level of toxicity (20% mortality) against the *S. litura* larvae. The comparisons between 72 and 96 hours data was highly significant for *C. dentata*

Table 2. Impact of methanolic extract and their Nano particless on the larval mortality (%) of *Spodoptera litura* larvae exposed after 24, 48, 72 and 76 hrs. Profit analyses were carried out for 96 hours data.

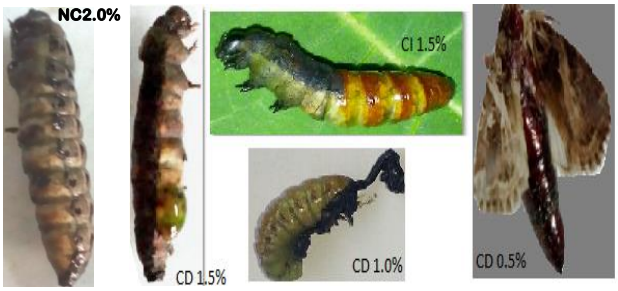
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**	**Profit Analyses for 96hrs**
			Control		
Concentrations (%)	18.52±8.47	25.92±12.86	40.74±14.41	48.14±15.19	
			C. dentata		
0.5	0.00±0	7.41±3.24	11.11±4.07	11.11±9.07	
1.0	0.00±0	7.41±3.24	11.11±4.07	14.81±5.24	LC_{50}=0.765%
1.5	0.00±0	14.81±5.24	22.22±4.07	25.92±12.86	χ^2 = 2.336
2.0	3.70±1.24	18.52±8.47	25.92±12.86	25.92±12.86	*P* = 0.05
			C. interruptus		
0.5	0.00±0	0.00±0	7.41±3.24	11.11±0	
1.0	0.00±0	7.41±3.24	7.41±3.24	14.81±5.24	LC_{50}= 0.890%
1.5	3.70±1.24	7.41±3.24	11.11±0	18.52±8.48	χ^2 = 3.469
2.0	7.41±3.24	7.41±3.24	18.52±8.48	18.51±8.48	*P* = 0.023
			N. cordifolia		
0.5	0.00±0	0.00±0	7.41±3.24	7.41±5.24	
1.0	0.00±0	0.00±0	7.41±3.24	11.11±4.07	LC_{50}= 0.9177%
1.5	0.00±0	3.70±1.24	7.41±3.24	11.11±4.07	χ^2 = 0.5480
2.0	3.70±1.24	7.41±3.24	11.11±4.07	14.81±8.87	*P*= 0.01
			Silver Nano particles (150µl conc.)		
CD-AgNPs	0.00±0	0.00±0	0.00±0	3.70±1.24	
CI-AgNPs	0.00±0	0.00±0	3.70±1.24	3.70±1.24	
NC-AgNPs	0.00±0	0.00±0	0.00±0	0.00±0	
Control ($AgNO_3$)	0.00±0	0.00±0	3.70±1.24	3.70±1.24	

0.00 -Indicates no mortality was observed

($df_{1,12}$, F=41.415, *P*=0.008) followed by *N. cordifolia* ($df_{1,12}$, F=72.079, *P*=0.0003). Whereas in case of *C. interruptus* the data was significant between 72 and 96 hours ($df_{1,12}$, F=10.500, *P*=0.05) than 24 and 96 hours ($df_{1,12}$, F=24.276; *P*=0.01).

Methanolic extract treated categories recorded the lowest percentage of adult emergence. No adults emergence was observed in *N. cordifolia* treated experimental category of *S. litura*. On the other hand *C. dentata* (0.5%) treated category recorded 11.11% followed by *C. interruptus* (0.5% and 1.5%) 22.22% as against 55.56% of adult emergence recorded in the control (Table 3). The developmental abnormalities observed in the experimental *S. litura* larvae such as the larval cadavers with cuticular rupture and leakage of body fluid (Plate 1), life stages of *S. litura* shrunken dark cadavers with remnants of moult skin, translucent body, larval pupal intermediate with improperly hardened pupal case with remnants of larval appendages (Plate 1) showed the impact of the experimental fern extracts on the normal development of the experimental animals.

Plate 1. Impact of chosen ferns *Christella dentata* (CD), *Cyclosorous interruptus* (CI) and *Nephrolepis cordifolia* (NC) on *Spodoptera litura*



The adults which emerged out from the treated categories of the experiment also showed improper metamorphosis. The adults that emerged from the methanolic extract categories had crumbled and deformed wings. They also retained the pupal case (Plate 1). In the present study the methanolic extracts and AgNPs of the experimental ferns resulted in moulting disruption, morphological anomalies and mortality in *S. litura* and it showed a dose-

Table 3. Impact of methanolic extract and their nano particles on biological traits- pupation, pupal mortality and adult emergence (%) of *Spodoptera litura*

Concentration (%)	Pupation	Pupal mortality	Adult emergence
Control	77.78 ± 7.02	22.22±3.21	55.56 ±5.91
C. dentate			
0.5	66.67±6.38	55.56±5.91	11.11±0.64
1.0	55.56±5.91	55.56±5.91	-
1.5	22.22±3.21	22.22±3.21	-
2.0	22.22±3.21	22.22±3.21	-
C. interruptus			
0.5	66.67±6.38	44.44±4.36	22.22± 3.21
1.0	55.56±5.91	55.56±5.91	-
1.5	44.44±4.36	22.22±3.21	22.22 ±3.21
2.0	44.44±4.36	44.44±4.36	-
N. cordifolia			
0.5	77.78±7.02	77.78±7.02	-
1.0	66.67±6.38	66.67±6.38	-
1.5	66.67±6.38	66.67±6.38	-
2.0	44.44±4.36	44.44±4.36	-
Silver Nano particles (150µl conc.)			
CD-Ag NP	77.78±7.02	77.78±7.02	-
CI-Ag NP	77.78±7.02	77.78±7.02	-
NC-Ag NP	88.89±7.99	-	-
Control Ag NO_3	77.78±7.02	77.78±7.02	-

dependent response. All the fern extracts revealed a developmental disruption in which the insects died in pharate condition following initiation of apolysis, but before completion of ecdysis. The insect moulting cycle is visibly initiated when the cuticular epithelium separates from the overlying cuticle. Moreover, the newly moulted *S. litura* larvae died while shedding the old cuticle and head capsule (pharate condition) (Plate 1). Similar findings were also reported by Muraleedharan (1988); Rajkumar *et al.* (2000); Sahayaraj and Paulraj (2000) and Sahayaraj *et al.* (2003) in lepidopteran pests such as *Achea janata, H. armigera* and *S. litura.* These developmental deformities could be due to the presence of analougues of ecdysones in ferns as stated by Selvaraj (2002). Hence the findings of the present experiment could be considered as significant evidence for the presence of phytoecdysone in the experimental ferns.

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