

## POTENTIATION OF *SYNEDRELLA NODIFLORA* L. FOR INSECTICIDAL ACTIVITY, INSECT REPELLENCY AND BRINE SHRIMP LETHALITY IN THE LABORATORY CONDITIONS

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**Abstract:** The insecticidal activity, insect repellency and brine shrimp lethality tests of three solvent extracts of *Synedrella nodiflora* L. were assessed against the adults of *Tribolium castaneum* (Herbst) under laboratory conditions. The methanol extract found most effective (LD<sub>50</sub> 1.229, 0.823 and 0.569mg cm<sup>-2</sup> for 24, 36 and 48h of exposure respectively) followed by the chloroform extract (LD<sub>50</sub> 3.042, 2.773, 2.456 and 2.233mg cm<sup>-2</sup> for 12, 24, 36 and 48h of exposure respectively) and petroleum ether extract (LD<sub>50</sub> 8.416, 4.764, 3.677, 3.392 and 3.177mg cm<sup>-2</sup> for 12, 24, 36, 48 and 60h exposure respectively). The CHCl<sub>3</sub> and MeOH extracts showed repellent activity against *T. castaneum* adults at P<0.05 and P<0.01 levels of significance, while the Pet. E. extract did not show any repellency. The similar extracts responded through brine shrimp lethality assay and the Pet. E. extract found most effective (LC<sub>50</sub> 321.182, 248.151, 182.196 and 140.866ppm) followed by the MeOH extract (LC<sub>50</sub> 428.356, 72.401, 68.397 and 22.161ppm for 12, 18, 24 and 30h of exposure respectively) and the CHCl<sub>3</sub> extract (LC<sub>50</sub> 669.875, 416.605, 288.226 and 248.325ppm). The results show the potential of using the *S. nodiflora* extracts for *T. castaneum* suppression.

**Keywords:** Dose-mortality, repellency, *Tribolium castaneum*, brine shrimp lethality, *Artemia salina*, *Synedrella nodiflora*,

**সারংশঃ** *Synedrella nodiflora* L. –এর তিন ধরনের নির্ধারিত পোকামাকড় দমন, পোকামাকড় বিতাড়ন ও brine shrimp এর উপর বিষ-কার্যকারিতা যাচাইয়ের জন্য চাউলের কেড়িপোকা *Tribolium castaneum* (Herbst) এর পূর্ণাঙ্গ দশার উপর গবেষণাগার ব্যবস্থায় পরীক্ষা চালান হয়। MeOH নির্ধারিত সবচেয়ে বেশী কার্যকর পাওয়া যায় (যেখানে ২৪, ৩৬, ও ৪৮ ঘন্টা প্রয়োগকৃত সময়কালের জন্য প্রতি বর্গসেন্টিমিটারে LD<sub>50</sub> ছিল যথাক্রমে ১.২২৯, ০.৮২৩ ও ০.৫৬৯ মি.গ্রা.), এরপর কার্যকর ছিল CHCl<sub>3</sub> নির্ধারিত (যেখানে ১২, ২৪, ৩৬, ও ৪৮ ঘন্টা প্রয়োগকৃত সময়কালের জন্য প্রতি বর্গসেন্টিমিটারে LD<sub>50</sub> ছিল যথাক্রমে ৩.০৪২, ২.৭৭৩, ২.৪৫৬ ও ২.২৩৩ মি.গ্রা.); তারপর স্থান পেয়েছে Pet. E. নির্ধারিত কার্যকারিতা (যেখানে ১২, ২৪, ৩৬, ৪৮ ও ৬০ ঘন্টা প্রয়োগকৃত সময়কালের জন্য প্রতি বর্গসেন্টিমিটারে LD<sub>50</sub> ছিল যথাক্রমে ৮.৪১৬, ৪.৭৬৪, ৩.৬৭৭, ৩.৩৯২ ও ৩.১৭৭ মি.গ্রা.)। পূর্ণাঙ্গ *T. castaneum* এর উপর CHCl<sub>3</sub> ও MeOH নির্ধারিত বিতাড়ন কার্যকারিতা প্রদর্শন করেছে যথাক্রমে P<০.০৫ ও P<০.০১ তাৎপর্যতায়, অবশ্য Pet. E. নির্ধারিত কোনরূপ কার্যকারিতা দেখায়নি। এই একই নির্ধারিতগুলি brine shrimp এর বিপরীতে বিষক্রিয়া দেখিয়েছে। এক্ষেত্রে Pet. E. নির্ধারিত ছিল সবচেয়ে বেশী কার্যকর (যেখানে ১২, ১৮, ২৪ ও ৩০ ঘন্টা প্রয়োগকৃত সময়কালের জন্য LC<sub>50</sub> ছিল ৩২১.১৮২, ২৪৮.১৫১, ১৮২.১৯৬ ও ১৪০.৮৬৬ ppm; এরপর কার্যকর ছিল MeOH নির্ধারিত (LC<sub>50</sub> ছিল ৪২৮.৩৫৬, ৭২.৪০১, ৬৮.৩৯৭ ও ২২.১৬১ ppm; সবশেষে CHCl<sub>3</sub> নির্ধারিতের জন্য LC<sub>50</sub> ছিল ৬৬৯.৮৭৫, ৪১৬.৬০৫, ২৮৮.২২৬ ও ২৪৮.৩২৫ ppm)।

### Introduction

*Synedrella nodiflora* L. (= *Verbesina nodiflora* L.) of the family Asteraceae is found in tropical Africa, Asia and West Indies. It is a perennial herb with prostrate stem, often pilose at the nodes with short trichomes to glabrate. Leaves 0.5-1.3x0.5-1.1cm, broadly ovate to orbicular, petioles 1-6mm long, apex rounded to slightly emerginate, base rounded to subcordate, globular puberulent beneath. Flower 1-2 in axil, rarely more, up to 5mm high, pedicels 2-6mm long. Sepals elliptic ovate to elliptic oblong, pubescent, climate corolla broadly campanulate, up to 10mm wide white. Fruit a globose capsule, 3-4mm across often reflex at maturity, seeds 1.5mm long, brownish to black subglobose. Flowering and fruiting throughout the year. It is traditionally used in Ghana for the treatment of epilepsy (Woode 2011). In Indonesia the leaves are used as a poultice for sore legs and rheumatism, and juice of leaves used for earache (Rathi and Gopalkrisnan 2005). In Fiji, a decoction of the leaves is used to treat haemorrhoids and diarrhoea. A decoction of the pounded and cooked roots is drunk as a cough-

mixture in Africa and in Barbados (Burkill 1985). *S. nodiflora* were highly biocidal in nature when they were tested on the crop plants like mustard and radish (Ghayal et al. 2008). Patrick et al. (2012) reported the neuropharmacological effects of the *S. nodiflora* extract. The present investigation was carried out to find out the potential of its insecticidal and repellent activity against the red flour beetle, *Tribolium castaneum* (Herbst), and lethality against the brine shrimp, *Artemia salina* L. nauplii. The red flour beetle is reddish-brown in color and its antennae end in a three-segmented club (Bousquet 1990). Although small beetles, about ¼ of an inch long, the adults are long-lived and may live for more than three years (Walter 1990), and thus became a suitable lab insect. The *Artemia salina* belong to a genus of very primordial crustacean (crawfish-crayfish) the *Anostraca* (Fairy Shrimps). Crawfish of this genus just have a divided exoskeleton made of chitin enhanced protein, no usual crust of chitin (escutcheon) as other crawfish have. There are many species within the genus of *Anostraca*, but the *A. salina* are very nice to grow, since the rate of successful hatches is very high.

## Materials and Methods

**Collection and preparation of test materials:** *S. nodiflora* was collected from the Rajshahi University Campus and identified by Prof. A.T.M. Naderuzzaman and a voucher specimen (No. 48, 10-12-1968) was deposited in the herbarium of the Department of Botany, University of Rajshahi. The plants were chopped into small pieces, dried under shade and powdered using a hand grinder, weighed and placed in separate conical flasks to add Pet. E.,  $\text{CHCl}_3$  and MeOH (Merck, Germany) (100gm  $\times$  300ml  $\times$  2times) for 48h. Filtration was done by Whatman filter paper (made in USA) at 24h interval in the same flask followed by evaporation until the extract were left. The extracts was then removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

**Collection and culture of test insect:** Adults of *T. castaneum* were reared in glass beakers (500ml) in a standard mixture of whole-wheat flour (ref) with powdered dry yeast (19:1) in an incubator at 30  $\pm$  0.5°C without light and humidity control for continuous supply of adults during experimentation.

**Dose-mortality test:** The dose-mortality responses of *S. nodiflora* were observed by surface film method. The concentrations used were 3.57, 3.05, 2.54, 2.04 and 1.52mg cm<sup>-2</sup> for Pet. E., 3.57, 3.05, 2.54, 2.04, 1.52 and 1.02mg cm<sup>-2</sup> for  $\text{CHCl}_3$ , and 2.54, 2.04, 1.52, 1.02, 0.52 and 0.25mg cm<sup>-2</sup> for MeOH extract. Each of the doses were diluted in 1ml of solvent, poured into Petri dishes and allowed to dry. Ten adult beetles were released in each Petri dish, and the experiment of all the doses for each of the extracts were replicated three times. The mortality of the beetles was assessed after 12, 24, 36, 48 and 60h of exposures.

**Statistical analysis:** The mortality (%) was corrected using Abbott's formula (1925):

$$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$$

Where,  $P_r$  = Corrected mortality (%),  $P_o$  = Observed mortality (%),  $P_c$  = Control mortality (%). The data were then subjected to probit analysis according to Finney (1947) and Busvine (1971) using a software developed at the University of Newcastle upon Tyne, UK.

Table 1 LD<sub>50</sub> values of Pet.E.,  $\text{CHCl}_3$  and MeOH extracts of *S. nodiflora* against *T. castaneum* adults.

Solvent	LD <sub>50</sub> at different hours				
	12	24	36	48	60
Pet. E.	8.416	4.767	3.677	3.392	3.177
$\text{CHCl}_3$	3.042	2.773	2.456	2.233	All dead
MeOH	-	1.229	0.523	0.569	All dead

**Repellent effects:** The  $\text{CHCl}_3$  and MeOH extracts of *S. nodiflora* offered a promising repellent effect against *T. castaneum* adults ( $P < 0.05$  and  $P < 0.01$  respectively) while the Pet. E. extract did not show any repellency (Tables 2 and 3).

**Repellent activity:** The repellency test was adopted from the method (No. 3) of McDonald et al. (1970) with some modifications. Half filter paper discs (Whatman No. 40, diameter 9 cm) were treated with the selected doses of 0.079, 0.039, 0.020, 0.010 and 0.005mg cm<sup>-2</sup> for Pet. E. extract and were then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in the Petri dishes. The orientation was changed in the two remaining replicates to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Ten adult insects were released in the middle of each of the filter paper circles. The similar process was done for the  $\text{CHCl}_3$  and MeOH extracts respectively.

Each concentration of each solvent was tested for five times. Insects that settled on each of the non-treated half of the filter paper discs were counted after 1h and then observed repeatedly at hourly intervals for five hours. The average of the counts was converted to percent repellency ( $PR$ ) using the formula of Talukder and Howse (1993, 1995):  $PR = (N_c - 5) \times 20$ , where,  $N_c$  is the percentage of insects on the untreated half of the disc.

**Brine shrimp nauplii lethality test:** Brine shrimp eggs were purchased from Kalabagan, Dhaka and kept in aerated seawater at room (25-30°C) temperature and took 30-48h to give nauplii. The series of concentration were 499.3, 249.6, 124.8, 62.5 and 31.2ppm for Pet. E., 499.5, 249.7, 124.8, 62.5 and 31.5ppm for  $\text{CHCl}_3$  and 600, 300, 150, 75 and 37.5ppm for MeOH extract. Ten freshly hatched nauplii were added to each of the test tubes with different concentrations mentioned earlier and observed mortality after 6, 12, 18, 24 and 30h of exposures. The data was then subjected to probit analysis.

## Results and Discussion

**Dose mortality effects:** The dose-mortality assay of Pet. E.,  $\text{CHCl}_3$  and MeOH extracts are represented in Table 1. The MeOH extract offered highest mortality giving LD<sub>50</sub> values ranged between 1.229 to 0.569mg cm<sup>-2</sup> followed by the  $\text{CHCl}_3$  extract ranged between 3.042 to 2.233mg cm<sup>-2</sup> and Pet. E. extract ranged between 8.416 to 3.177mg cm<sup>-2</sup>.

Table 2 Percent repulsion values and the arcsin transformed data of the Pet. E., CHCl<sub>3</sub> and MeOH extracts of *S. nodiflora* against *T. castaneum* adults.

Type of extract	Dose cm <sup>-2</sup> mg	Percent repulsion PR = (Nc - 5) × 20 in hours (Arcsin transformed values for ANOVA)				
		1	2	3	4	5
Pet.E	10	40 (39.23)	66.6 (54.70)	73.2 (58.82)	73.2 (58.82)	80 (63.43)
	5	100 (90)	100 (90)	93.2 (74.88)	86.6 (68.53)	100 (90)
	2.5	100 (90)	93.2 (74.88)	93.2 (74.88)	86.6 (68.53)	80 (63.43)
	1.25	86.6 (68.53)	100 (90)	66.6 (54.70)	86.6 (68.53)	93.2 (74.88)
	0.625	73.2 (58.82)	86.6 (68.53)	86.6 (68.53)	53.2 (48.83)	60 (50.77)
CHCl <sub>3</sub>	10	93.2 (74.88)	100 (90)	100 (90)	100 (90)	100 (90)
	5	80 (63.43)	73.2 (58.82)	73.2 (58.82)	73.2 (58.82)	73.2 (58.82)
	2.5	60 (50.77)	-33.4 (35.30)	73.2 (58.82)	-13.4 (21.39)	-40 (39.23)
	1.25	-6.6 (4.66)	6.6 (4.66)	-13.2 (21.30)	-20 (26.57)	-20 (26.57)
	0.625	-26.8 (31.18)	-33.4 (35.30)	-40 (39.23)	-93.2 (74.88)	-73.4 (58.95)
MeOH	10	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)
	5	-53.2 (46.83)	-73.4 (58.82)	-53.2 (46.83)	-53.2 (46.83)	-53.2 (46.83)
	2.5	-20 (26.57)	-26.8 (31.18)	-33.4 (35.30)	-33.4 (35.30)	-20 (26.57)
	1.25	-53.2 (46.83)	-46.8 (43.17)	26.6 (31.05)	26.6 (31.05)	00 (00)
	0.625	-53.2 (46.83)	-46.8 (43.17)	-73.4 (58.95)	-73.4 (58.95)	-60 (50.77)

Table 3 Repellency effect of the Pet. E., CHCl<sub>3</sub> and MeOH extracts of *S. nodiflora* against *T. castaneum* adults.

Solvent	Between doses (df=4)	Between time interval (df=4)
	F-value	F-value
Pet. E.	1.043	5.95
CHCl <sub>3</sub>	0.883	22.29*
MeOH	1.083	24.85**

**Brine shrimp lethality effect:** The brine shrimp lethality for Pet. E., CHCl<sub>3</sub> and MeOH extracts of *S. nodiflora* represented in Table 4. The Pet. E. extract offered the highest activity with LC<sub>50</sub> values 321.182 to 140.866ppm, followed by the MeOH extract with LC<sub>50</sub> values 428.356 to 22.161ppm and for the CHCl<sub>3</sub> extract with LC<sub>50</sub> values 669.875 to 248.325ppm all for 12, 18, 24 and 30h of exposures respectively.

Table 4 LC<sub>50</sub> values of Pet. E., CHCl<sub>3</sub> and MeOH extracts of *S. nodiflora* against *A. salina* nauplii.

Solvent	Duration of exposure in hours			
	12	18	24	30
Pet.E	321.182	248.151	182.196	140.866
CHCl <sub>3</sub>	669.875	416.605	288.226	248.325
MeOH	428.356	72.461	68.397	22.161

These findings receive supports from previous researchers' achievements. The whole plant extract of the *S. nodiflora* has shown antioxidant and cytotoxic activity (Dutta et al. 2012). Insecticidal activity of aerial parts of *S. nodiflora* on *Spodoptera litura* has been reported by Rathi and Gopalakrishnan (2005). Extracts of *S. nodiflora* (L.) Gaertn. displayed anti-inflammatory, analgesic, antinociceptive, antipyretic and insecticidal properties (Abad et al. 1996; Forestieri et al. 1996; Rathi and Gopalakrishnan 2006; Woode et al. 2009). Bhogaonkar (2011) mentioned that *S. nodiflora* (L.) Gaertn. is a less known medicinal plant and it has good antibacterial and antifungal activity. Traditionally, the leaves are used as poultice for rheumatism and the juice of the leaves is used for earache in India (Rathi and Gopalakrishnan 2006). The ethanol extract of *S. nodiflora* has antioxidant and antibacterial properties on account of phenolic compounds of flavonoids (Sumi 2011). The hydro-ethanolic extract of *S. nodiflora* exhibits anxiogenic-like effects which could be attributed to sedation and reduction in locomotor activity (Woode et al. 2011). Thus, a comprehensive phytochemical analysis of the plant for its insecticidal and repellent components, as well as the physiological studies of the active ingredients are very much to be solicited for their effective use in the future pest control and pharmaceutical endeavors.

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