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DETERMINATION OF LETHAL CONCENTRATION OF TROPICAL SODA APPLE, *SOLANUM VIARUM* (DUNAL) AGAINST COMMON CUTWORM, *SPODOPTERA LITURA* (FABRICIUS) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Spodotera litura a polyphagous pest on economically important crops such a cotton, groundnut, chilli, tobacco, bhendi and pulses are developing resistant to most of the insecticides available in the market and in order to develop an alternate method. The present study targets to workout determent dose 50 for *Solanum viarum* fruit extract by following feeding assay against *S.litura.* Three types of extracts were used in the feeding assay to experiment insecticidal activity of *S.vairum* fruit extract. In total 10 accessions and one variety of *S.viarum* fruits were tested for their insecticidal effects. Among the tested accessions kolli hills accessions were found effective in exerting mortality. The LC_{50} worked out 0.48% for the *S.viarum* fruits (kolli hills accessions)

Keywords: *Solanum viarum,* determent dose, *Spodoptera litura,* anti-feedant activity.

1.INTRODUCTION

Solanum viarum is a member of the economically important family Solanaceae which consisting of 75-90 genera and more than 2,500 species of plants. Many of these are used as ornaments, food and drugs (Mullahey *et al.,* 1993). Besides the common name "tropical soda apple". *S. viarum* is originally native to Argentina and Brazil (Nee, 1991) and spreaded into other parts of South and central America and has been introduced in the Caribbean, West Indies, Nepal, India and Africa (Coile, 1993 and Wunderlin *et al.,* 1993). Fruits of *S. viarum* are globular, glabrous, about 2-3 cm in diameter and yellow in colour when mature. Most distinctive is the immature fruit, which is green with white mottling, similar in appearance to immature water melon fruit (Mullahey and Colvin, 1993). Freshly opened fruit has a sweet odour similar to a plum or apple but the seeds have a bitter taste, however they are not sufficiently detractive to wildlife and cattle which actively consume the fruit and thus spread the seed (Mullahey, 1996). Insect pests are known to productivity. *Spodoptera litura* is an important polyphagous pest in India, China and Japan. It is serious pest of various economically important crops such as cotton, groundnut, chilli, tobacco, castor, bhendi and pulses etc., (Armes *et al*., 1997; Niranjankumar and Ragupathy, 2001).In this study, insecticidal properties of *S.viarum* was tested against *S.litura*

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2.MATERIALS AND METHODS

Rearing of *Spodoptera litura*

Tobacco caterpillar, *Spodoptera litura* (Noctuidae: Lepidoptera), reared on Bengal gram flour based semi synthetic diet (PDBC, 1998) under the temperature of 25 ± 2 ^{o}C and 70±5 per cent relative humidity continuously throughout the study period in our laboratory, was utilized in the anti-feedant bioassays as test insect.

Collection of various ecotypes and accessions of *Solanum viarum*

Solanum viarum fruits were collected from different places in Tamil Nadu *viz.,* Annamalainagar (Cuddalore District), Kolli hills, Semmadu village, Separapakkam village and Arapalliswarar temple (Namakkal District) and Pachamalai. Ponavarai village, Oodaikattupudur village, Pariyapakklam and Nasakulam village (Trichy District) for extraction. Yellowish ripened fruits were collected and placed in cloth bag and brought to the laboratory. In total, 10 accessions

and one variety (six accessions from Kollihills and four from Pachamalai and a variety *Arka sanjeevini* from Annamalainagar) were used in extraction.

Extraction of *Solanum viarum* fruits

Fruits of *S. viarum* collected from Kollihills (6 Accessions), Pachamalai hills (4 Accessions) and Annamalainagar (Variety: *Arka Sanjeevini*) were extracted using various solvents such as n-hexane, benzene, ethyl acetate, acetone and combinations of solvents at different ratio *viz.,* n-hexane and benzene (50:50), benzene and ethyl acetate (50:50) and ethyl acetate and acetone (50:50). Three methods such as Soxhlet extraction method, Cold extraction method (room temperature extraction method) and Aqueous extraction method had employed in extracting fruits by using solvents.

Soxhlet extraction method

The fruits were cut and packed as 50 g packets using Whatman No. 40 filter paper and extracted with n-hexane, benzene, ethyl acetate, acetone, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50), ethyl acetate and acetone (50:50) (HPLC grades) for 72 hrs., in a Soxhlet apparatus individually. Then extracts were passed through anhydrous sodium hydroxide to absorb water particles and dried under reduced pressure using vacuum pump. Finally the extracts were labeled and stored in a defreezer (-10 ºC).

Cold extraction method (Room temperature extraction method)

Cut pieces of fruits packed as 250 g packets using Whatman No. 40 filter paper were used in extraction. Packets were placed in round-bottom (5 lit. capacity) stopper flasks and added with respective solvent [n-hexane, benzene, ethyl acetate, acetone, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50), ethyl acetate and acetone (50:50) (HPLC grades)]. After 72 hrs., the paper packets were removed from the flasks and the extracts were passed through anhydrous sodium hydroxide to absorb water particles and dried under reduced pressure using vacuum pump. Then the extracts were labeled and stored in a deep freezer (-10ºC).

Aqueous extraction method

Cut pieces of berries packed as 250 g packets using Whatman No. 40 filter paper were extracted with HPLC grade water at room temperature in round-bottom (5 lit., capacity) stopper flasks. After 72 h, the paper packets were removed from the flasks and the extracts were labeled and stored in a refrigerator.

Poison food bioassay (Leaf disc bioassay)

Bio-assay was conducted to select effect accessions and effective extract

Castor leaf discs of 2 cm diameter were cut and the respective extract was smeared on the both the adaxial and abaxial surfaces of the leaf disc @ 100 µl/side using a blunt glass rod. The concentration tested was 1%. Untreated leaf discs served as absolute control. Treated leaf discs were air dried and placed in petriplates. Three larvae of fourth instars of *S. litura* were introduced separately in to each petriplate and covered. All the petriplates were kept under controlled conditions of 25 ± 2ºC temperature and 70 ± 5 % relative humidity. Leaves were collected from the container when the control leaf disc was completely fed and the leaf area unfed was measured in each treatment by using leaf area meter (Elico model). Per cent leaf area protection over control was computed and the antifeedancy was rated as per the formula and scale given below. The larvae were supplied with untreated leaves and reared till the life cycle ends. Observations were also made on mortality and malformations in any of its life stages. In total 15 treatments were followed and each treatment was replicated three times. Based on the observations, promising extract of *S. viarum* fruit were identified (Arivudainambi, 2001).

Per cent leaf area protection over control =

$$\frac{\text{Per cent of leaf area protection in treatment} - \text{Per cent of leaf area protection in absolute control}}{100 - \text{Per cent of leaf area protection in absolute control}} \times 100$$

Determination of lethal concentrations of effective extract against *Spodoptera litura*

Lethal concentrations were determined for the effective accessions and extract selected in the above bioassay.

$LC_{30,}$ LC_{50} and LC_{90} values of the effective extract of *S. viarum* fruits was worked out against the fourth instar of *S. litura* by following the leaf disc bioassay technique by using five concentrations (0.2, 0.4, 0.6, 0.8, 1.0 per cent) along with absolute control. Each treatment was replicated five times. Fifteen larvae were used

per treatment and data on the number of dead larvae was recorded at 48 hrs., post treatment. Mortality was corrected using Abbott's formula (1925) if mortality observed in absolute control. $LC_{30,}$ LC_{50} and LC_{95} values and fiducial limits were worked out by following probit analysis (Finney, 1971).

Statistical analysis

All the percentage data were subjected to arc - sine transformation and whole numbers were log transformed. Lethal concentrations were worked out by using probit analysis. Analysis was done with ANOVA and the means were compared by following Duncan's multiple range test (DMRT) at p = 0.05 (Gomez and Gomez, 1984).

3.RESULTS

Poison food bioassay (Leaf disc bioassay) of Soxhlet, Cold and Aqueous fruit extraction of *Solanum viarum* against LC_{50} of *Spodoptera litura*

Acetone extracts of *S. viarum* accessions *viz.,* KH-3, KH-4, KH-5 and KH-6 recorded 93.33% mortality against fourth instar *S. litura* at 1% concentration. This was followed by acetone extracts of accessions KH-1 and KH-2, where 86.66% mortality was observed. Acetone extracts of Pachamalai accessions exerted 46.66 to 66.66 per cent mortality. Among the combinations of solvents used for extraction, ethyl acetate and acetone combination at 50:50 showed better performance than control. The mortality range falls between 10 to 20% and 3.33 to 10.00 in KH and PH accessions respectively in the above combination. All the

accessions extracted using benzene and ethyl acetate recorded zero per cent morality in all the replications. Untreated also recorded no mortality in any of the replications observed. A variety *Arka sanjeevini* showed the maximum of 20 per cent mortality while extracted using acetone and it was followed by T7 the combinations of ethyl acetate and acetone at the ratio of 50:50 (Table 1).

The accessions extracted by following cold solvent method showed a different result in exerting mortality. Acetone extracts (T4) recorded the maximum per cent mortality among all the treatments and within the treatment the accessions KH-2 and KH-3 reported cent percentage morality. The other accessions of Kolli hills such as KH-1, KH-4, KH-5 and KH-6 recorded 93.33% mortality. Pachamali hills accessions PH-1, PH-2, PH-3 and PH-4 showed the mortality of 73.33, 53.33, 53.33 and 46.66% respectively. *Arka sanjeevini* the released variety recorded 26.66% mortality while extracted with acetone. Other solvents used in extraction of fruits did not record considerable mortality (Table 2).

Table 1. Efficacy of fruit extracts (Soxhlet Extraction Method) of *S. viarum* accessions on fourth instar of *S. litura* – Poison food bioassay

T. no.	Treatment	*Per cent mortality at 1% concentration										
		KH-1	KH-2	KH-3	KH-4	KH-5	KH-6	PH-1	PH-2	PH-3	PH-4	AS
1.	n-hexane extract	6.66 (8.85)^{bc}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
2.	Benzene extract	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
3.	Ethyl acetate extract	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
4.	Acetone extract	86.66 (72.28)^{a}	86.66 (72.28)^{a}	93.33 (81.14)^{a}	93.33 (81.14)^{a}	93.33 (81.14)^{a}	93.33 (81.14)^{a}	66.66 (54.99)^{a}	60.00 (50.77)^{a}	46.66 (43.07)^{a}	53.33 (46.92)^{a}	20.00 (26.50)^{a}
5.	n-hexane and benzene (50:50) extract	6.66 (12.28)^{bc}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	6.66 (12.28)^{b}
6.	Benzene and ethyl acetate (50:50) extract	3.33 (6.14)^{c}	0.00 (0.00)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	3.33 (6.14)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
7.	Ethyl acetate and acetone (50:50) extract	16.66 (23.85)^{b}	20.0 (26.57)^{b}	13.33 (21.14)^{b}	10.00 (18.43)^{b}	13.33 (21.14)^{b}	13.33 (21.14)^{b}	10.00 (18.43)^{b}	3.33 (6.14)^{b}	3.33 (6.14)^{b}	3.33 (6.14)^{b}	10.00 (18.43)^{b}
8.	Untreated check	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
#9-15.	Solvent controls	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
	CD (0.05)	17.11	13.50	14.21	13.86	12.98	12.98	8.25	8.62	9.95	8.25	6.58

*Mean of three replications, Values in parentheses one arc sine transformed values, Values with different alphabets differ significantly
(# It includes solvent controls such as (T9-T15) n-hexane, benzene, ethyl acetate, acetone, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50) and ethyl acetate and acetone (50:50)ased variety);KH – Kolli hills accessions - PH – Pachamalai hills accessions - AS–*Arka sanjeevini*

Regarding the aqueous extract of *S. viarum* fruits, Kolli hills accessions 1, 4 and 6 reported 100 per cent mortality against *S. litura* and the accessions 2, 3 and 5 recorded 93.33% mortality. Pachamalai hills accession 4 was recorded 40% mortality and this was followed by accessions 1, 2 and 3. *Arka sanjeevini* the variety of *S. viarum* recorded 53.33% mortality. There was no mortality in untreated check (Table 3).

Table 2. Efficacy of fruit extracts (Cold Extraction Method) of *S. viarum* accessions On fourth instar of *S. litura* – Poison food bioassay

T. no.	Treatment	*Per cent mortality at 1% concentration										
		KH-1	KH-2	KH-3	KH-4	KH-5	KH-6	PH-1	PH-2	PH-3	PH-4	AS
1.	n-hexane extract	0.00 (0.00)^{c}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
2.	Benzene extract	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
3.	Ethyl acetate extract	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
4.	Acetone extract	93.33 (81.14)^{a}	100 (90.0)^{a}	100 (90.00)^{a}	93.33 (81.43)^{a}	93.33 (81.14)^{a}	93.33 (81.14)^{a}	73.33 (59.21)^{a}	53.33 (46.92)^{a}	53.33 (46.92)^{a}	46.66 (43.07)^{a}	26.66 (307.9)^{a}
5.	n-hexane and benzene (50:50) extract	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{bc}	3.33 (6.14)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	6.66 (12.28)^{b}
6.	Benzene and ethyl acetate (50:50) extract	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	33.33 (6.14)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
7.	Ethyl acetate and acetone (50:50) extract	20.10 (26.07)^{b}	16.66 (25.85)^{b}	10.00 (18.43)^{b}	10.00 (18.43)^{b}	16.66 (23.85)^{b}	13.33 (21.14)^{b}	6.66 (12.28)^{b}	3.33 (6.14)^{b}	3.33 (6.14)^{b}	3.33 (6.14)^{b}	6.66 (12.28)^{b}
8.	Un treated check	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
# 9-15	Solvent controls	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}
CD (0.05)		13.56	12.11	9.64	11.16	13.43	11.99	10.54	0.11	10.67	7.25	10.24

*Mean of three replications, Values in parentheses one arc sine transformed values, Values with different alphabets differ significantly (# It includes solvent controls such as (T9-T15) n-hexane, benzene, ethyl acetate, acetone, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50) and ethyl acetate and acetone (50:50)ased variety);KH – Kolli hills accessions - PH – Pachamalai hills accessions - AS–*Arka sanjeevini*

Table 4. Lethal concentrations of *S. viarum* fruit extract (KH-4 accession) against fourth instar of *S. litura* – Poison food bioassay

T.No.	Concentration (in 1%)	No. of Insects	No. of Dead	% larval mortality	Corrected % of mortality
1.	0.2	15	2	13.33	13.33
2.	0.4	15	5	33.33	33.33
3.	0.6	15	8	53.33	53.33
4.	0.8	15	12	80.00	80.00
5.	1.0	15	14	93.33	93.33
6.	Absolute Control	15	0	0.00	0.00

LC_{30} = 0.34

LC_{50} = 0.48

LC_{95} = 1.40

Slope (b) = 3.55686

Intercept (a) = -4.5456

Y = -0.4.55 + 0.3.56X

Table X2 = 7.81473

Calculated X2 = 1.84736

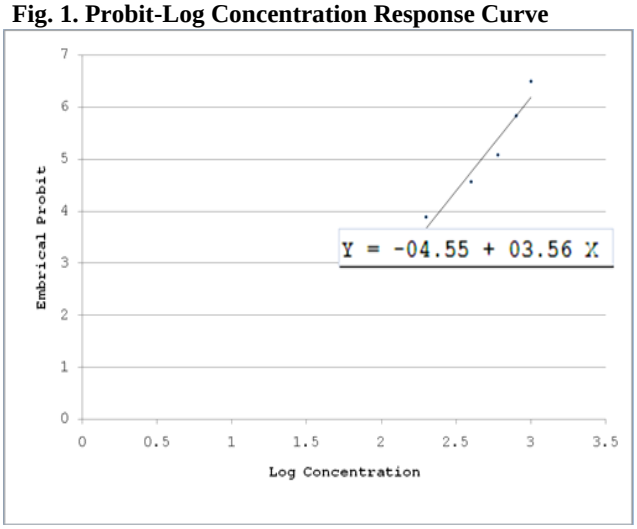
Table 3. Efficacy of fruit extracts (Aqueous Extraction Method) of *S. viarum* accessions on fourth instar of S. *litura* – Poison food bioassay

Tr. No.	Treatment	*Per cent mortality at 1% concentration
1.	*Solanum viarum* (KH1)	100.00 (90.00)[a]
2.	*Solanum viarum* (KH 2)	93.33 (81.14)[a]
3.	*Solanum viarum* (KH 3)	93.33 (81.14)[a]
4.	*Solanum viarum* (KH 4)	100.00 (90.00)[a]
5.	*Solanum viarum* (KH 5)	93.33 (81.14)[a]
6.	*Solanum viarum* (KH 6)	100.00 (90.00)[a]
7.	*Solanum viarum* (PH 1)	33.33 (35.01)[b]
8.	*Solanum viarum* (PH 2)	33.33 (35.01)[b]
9.	*Solanum viarum* (PH 3)	26.66 (35.79)[b]
10.	*Solanum viarum* (PH 4)	40.00 (38.85)[b]
11.	*Solanum viarum* (AS)	53.33 (46.92)[b]
12.	Untreated check	0.00 (0.00)[c]
	CD (0.05)	14.71

*Mean of three replications;Values in parentheses one arc sine transformed values; Values with different alphabets differ significantly; KH – Kollihills accessions; PH – Pachamalai hills accessions; AS – *Arka sanjeevini*

Kolli hills accessions No.4 was found as effective extract. Cold solvent and soxhlet methods were equally effective. So,LC_{50} was worked out for KH_4 fruit extreact (cold solvent method).Lethal concentration (LC_{50}) of KH (Kolli Hills) accession No.4 was 0.48%. (Table 4 & Fig. 1)

Fig. 1. Probit-Log Concentration Response Curve



4.DISCUSSION

Perusal of data on bioassays against larvae of *S. litura* indicated that Kollihills accessions showed good insecticidal property. All the six accessions, collected from Kollihills, exerted more than 86% mortality and even few registered 100% mortality. Pachamalai accessions recorded low insecticidal properties (46 % to 66%) when compared with Kollihills accessions. The variety *Arka sanjeevini* used in the study recorded very poor insecticidal property (20%) when compared with Koll ihills and Pachamalai accessions. The above said promising antifeedent activities were found in the treatments where acetone and water used as solvent in extraction. The solvents such as n-hexane, benzene, ethyl acetate, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50), ethyl acetate and acetone (50:50) were not found effective in extracting the active principle from the fruits. It implies that the active principle can be extracted by acetone and water alone. When comparing the extracts obtained by following Soxhlet, Cold and aqueous extraction methods, the results conveyed that cold extraction method is superior to soxhlet method and equal to aqueous extraction method.

Thus, fruits collected from Kolli hills accessions, extracted using acetone and water by following cold extraction and aqueous extraction methods respectively were found efficient when poison food bioassays were employed. Acetone and aqueous extracts of Kolli hills accessions were found effective against fourth instar *S. litura* at 1% concentration and their mortality ranges between 86 and 100%. This was seen only in poison food bioassay but same extracts at same concentration were not exerted significant insecticidal action in topical bioassay. This shows that the extract might not be a contact insecticide. Based on the bioassay results it is concluded that *S. viarum* fruit extract have toxic action on insects when ingested. Further it effective against the insects which belong to biting and chewing type. It is well established fact that different climatic, soil and seasonal conditions influence the quantity of the secondary metabolites of plants as shown by Aalbergberg and singh (1991) and Menut *et al.,* (1993). Similarly, rhizomes of *Acorus calamus* L., which is widely distributed in Asia, North America and Europe, contain β-a sarone a toxic and sterilizing chemical. However, β- a sarone content of plants growing in Asia was higher (70-96%) in contrast to the plants growing in North America and Europe (15%) (Schmidt and Streloke, 1994). Seenirangasamy *et al.* (1993) observed that the azadirachtin content of neem ecotypes varied according to the seasonal conditions. Ecotypes growing in extreme hot and cold climates showed very low azadirachtin content where as higher azadirachtin content was recorded at optimum growth conditions. Arivudainambi (2001) found that the anti-insect properties of stilt root extracts of *Rhizophora* sp., were not static and were greatly influenced by seasonal conditions. The stilt root collected during summer cause higher anti-insect properties than winter collected.

In the present study variations in antifeedent properties among the accessions of *S. viarum* may due to the variations of active principle content in the fruit. The reason for this variation in active principle content is obscure, because the climate and soil type are identical in kollihills and pachamalai. There is no deviation in the collection period (season) also. Our findings in relation to extraction procedure is in accordance with the statement of Jaglan *et al.* (1997) who reported that *Azadirachta indica A. Juss, Melia azedirach A. Juss* and *Lantana camara L.* extracted by following cold extract method was effective than sohlext method. Similarly, Arivudainambi (2001) reported that *C. collinus* extracted by following cold extraction method was effective against *S. litura* than soxhlet and aqueous methods.

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