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# Bioefficacy of Flindersine against *Helicoverpa armigera* Hübner, *Spodoptera litura* Fabricius, *Anopheles stephensis* Liston. and *Culex quinquefasciatus* Say.

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## ABSTRACT

*Flindersine, an alkaloid isolated from* Toddalia asiatica, *was evaluated for their antifeedant, larvicidal and growth inhibitory activities against* Helicoverpa armigera, Spodoptera litura *and larvicidal activity against vector mosquitoes* Anopheles stephensi *and* Culex quinquefasciatus. *For this, leaf disc no choice method was used for agricultural pests; larvicidal activity was tested on second and fourth instar larvae for mosquitoes at different concentrations. Flindersine showed antifeedant, larvicidal and growth inhibitory activities against* H. armigera and S. litura *and larvicidal activity against vector mosquitoes* An. stephensi *and* Cx. quinquefasciatus. *It showed high regression ($R^2$) values of 0.91 and 0.87 against* H. armigera *and* S. litura, *respectively for antifeedant activity. Flindersine exhibited more than 65% larvicidal activity against both the pests with $LC_{50}$ values of 443.04 and 568.88 ppm and $R^2$ values of 0.87 and 0.90 against* H. armigera *and* S. litura, *respectively. The larval and pupal duration of tested insects increased to more than five days at 1000 ppm when compared with the control. The adult emergence was reduced when the concentration of flindersine was increased. At 1000 ppm, no adult emergence was observed in both the pests. Flindersine exhibited 100% larvicidal activity against both the tested mosquitoes at 20 ppm concentration, which showed $LC_{50}$ values of 2.90, 4.19, 1.68 and 2.71 ppm for 2nd and 4th instar larvae of* Cx. quinquefasciatus *and* An. Stephensi, *respectively. High regression values were observed for antifeedant, larvicidal and growth inhibitory activities. Flindersine could be used to develop an ecofriendly pesticide formulation to control the agricultural pests and vector mosquitoes.*

**Key words:** Flindersine, Adult emergence, Antifeedant, Growth inhibitiory, Larvicidal

## INTRODUCTION

Chemical pesticides have been playing a major role in plant protection to increase the production. At the same time, they also cause severe adverse health effects on humans and animals. Indiscriminate use of synthetic pesticides in agricultural ecosystem causes environmental pollution, development of insect resistance and more importantly affects the non-target organisms; they also reduce the natural enemy populations (Youn et al. 2003). Hence, there is a need for ecofriendly pesticides. Bioesticides are considered an alternatives to chemical pesticides; they are ecofriendly and also possess many modes of action. Several studies have been made on

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plant-based pesticides. According to Balick et al. (1995) less than 1% of bioactive natural compounds have been chemically identified from plant source.

Many plant extracts, semi purified and purified compounds have been studied for insect control properties by many researchers all over the world. Secondary metabolites from plants play a key defensive role against the insects and act as antifeedants, oviposition deterrents and growth inhibitors (Isman 2002). Antifeedants are important factors in insect-plant interaction and are useful to study the field crop pests' behavior (Usher et al. 1988). Natural compounds such as Juglone, plumbagin and emodin showed antifeedancy against *Trichoplusia ni* (Hübner) (Akhtar et al. 2012). Plant derived compounds are toxic to insect pests; some of the compounds are highly active (Salehzadeh et al. 2002). Munoz et al. (2013) reported that *Calceolaria talcana* (Calceolariaceae) derived extracts revealed antifeedant, larvicidal, and growth inhibitory activities (reduced the adult emergence, pupal weight and pupation) against *S. frugiperda* (J.E. Smith). Ar-turmerone from *Curcuma longa* (Zingiberaceae) inhibited the growth (width of head capsule, length and weight) of *S. frugiperda* (Tavares et al. 2013). Rhodojaponin-III, a grayanoid diterpene from *Rhododendron molle* (Ericaceae) acted as antifeedant, stomach poison, contact toxicant and insect growth inhibitor against *Pieris rapae* Linnaeus (Zhong et al. 2006).

*Toddalia asiatica* from Rutaceae family has many medicinal properties. It is widely used as a folk medicine in India. The Kani tribes use the extract of this plant to cure skin diseases (Ayyanar and Ignacimuthu 2005). The extracts of this plant exhibited strong insecticidal properties against *Aedes aegypti* Linnaeus, *A. albopictus* Skuse and *Culex quinquefasciatus* Say (Borah et al. 2010; Liu et al. 2013; Nyahanga et al. 2013); and larvicidal and antifeedant properties against *Helicoverpa armigera* Hübner (Sundararajan and Kumuthakalavalli 2001; Ramya et al. 2009). *H. armigera* is a polyphagous pest causing serious damage to many agricultural and horticultural crops such as cotton, tomato, brinjal, chickpea, pigeonpea, chilli, maize, and sorghum (Manjunath et al. 1989; Talekar et al. 2006). Over 112 plant species from 44 families of plants are infested by this insect. In India, this is reported to feed on over 40 species (Ramana et al. 1988; Dhir et al. 1992). Larvae feed on the leaves, flowers and even pods (Kriti et al. 2014). *Spodoptera litura* Fabricius is a polyphagous pest. It also attacks more than 100 crops (Talukder and Howse 1994). Both *H. armigera* and *S. litura* have developed resistance against synthetic pesticides (Kumar and Reghupathy 2001; Ramasubramaniam and Regupathy 2004).

Mosquitoes are responsible for spreading diseases like malaria, Japanese encephalitis, yellow fever, dengue and filariasis. Among the diseases, *Cx. quinquefasciatus* spread the lymphatic filariasis which is more common in Indian subcontinent (Rajasekariah et al. 1991). Malaria is a deadly disease in India, which is spread by *An. stephensi*. Large numbers of programmes are conducted for mosquito larvicidal controls at the breeding sites, which are most common method in India to control and manage the mosquitoes. Fogging is the temporary method to control the adult mosquitoes,; the most efficient method is to manage the mosquito population by killing the larvae. Many researchers highlighted the effects of botanical pesticides on vector mosquitoes. Senthilkumar et al (2008) found that essential oil from *Blumea mollis* (Asteraceae) exhibited larvicidal activity on *Cx. quinquefasciatus*. Herbal formulation Ponneem exhibited larvicidal activity against *Aedes aegypti* Linnaeus and *A. albopictus* (Maheswaran and Ignacimuthu 2012). Benzene and their fractions of *Citrullus vulgaris* Cucurbitaceae exhibited larvicidal activity against *Anopheles stephensi* Liston and *A. aegypti* (Mullai et al. 2008). The present study was evaluated the antifeedant and toxic effects of flindersine isolated from *T. asiatica* against *H. armigera*, *S. litura*, *An. Stephensi* and *Cx. quinquefasciatus*.

## MATERIAL AND METHODS

### Plant material

The leaves of *Toddalia asiatica* Lam. (Rutaceae) were collected from the forest area of Kanchipuram District, Tamil Nadu, India. The plant was identified by a taxonomist at the Department of Botany, Madras Christian College, Chennai, India. The voucher specimen (ERIC-D-78) was deposited at the Entomology Research Institute, Loyola College, Chennai, India.

### Flindersine

The isolation and identification of flindersine (Fig. 1) was done following Duraipandiyan and Ignacimuthu (2009).

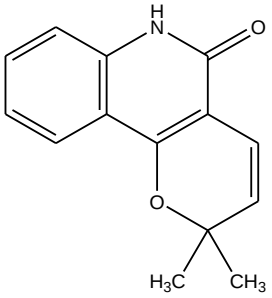


**Figure 1 -** Flindersine (2,6-dihydro-2,2-dimethyl-5H-pyrano [3,2-c] quinoline-5-one-9cl) isolated from *Toddalia asiatica* leaves.

**Insect culture**

Larvae of *H. armigera* and *S. litura* were collected from the farmers' field in Monnavedu, Thirvallur district of Tamil Nadu. *H. armigera* larvae were reared individually in a plastic container and fed regularly with bhendi fruit (*Abelmoschus esculentus* L.) (Malvaceae); *S. litura* larvae were offered castor leaves. The larvae were kept till they became pupae under the laboratory conditions (27 ± 2°C and 75 ± 5% relative humidity). Sterilized soil was provided for pupation. After pupation, the pupae were collected from the soil and placed inside the cage for the emergence of adults. Cotton soaked with 10% honey solution mixed with a few drops of multivitamins was provided for adult feeding to increase the fecundity. Potted cowpea plants were kept for *H. armigera*, and groundnut plants were provided for *S. litura* separately inside the adult emergence cages for egg laying. After hatching, the larvae were collected from the respective cages and fed with standard artificial diet as recommended by Koul et al. (1997) for *H. armigera* and castor leaf for *S. litura*.

**Rearing of vector mosquitoes**

Larvae of *An. stephensi* and *Cx. quinquefasciatus* were derived from various places with stagnant water bodies in Chennai, India. They were cultured in the laboratory for many generations and were maintained continuously at laboratory conditions (27 ± 2°C, 75–85% RH and photoperiod of 14:10h, light/dark). Full development from the egg to adult lasted for about 3–4 weeks. Larvae were fed on finely ground dog biscuit mixed with yeast extract in the ratio of 3:1. The water was changed daily to avoid scum formation. Pupae were transferred from the trays to a plastic cup containing tap water and placed inside the cage (30 x 30 x 30 cm dimension) for adult emergence. The emerged adults were reared in respective cages. The adult colony was provided with ten percent sucrose solution and it was periodically blood-fed on restrained rats. After three days, ovitrap was kept in the cages for egg laying and the eggs were collected and transferred to enamel trays. Newly hatched second and fourth instar larvae were used for the present experiments.

**Antifeedant activity**

Antifeedant activity of flindersine was studied using leaf disc no-choice method. Fresh cotton (*H. armigera*) and castor (*S. litura*) leaf discs of 4 cm diameter were punched using a cork borer. They were dipped in 125, 250, 500 and 1000 ppm flindersine and azadirachtin individually. The leaf discs dipped in acetone were used as negative control since acetone was used to dissolve the compound. In each plastic Petri dish (1.5 cm × 9 cm), a wet filter paper was placed to avoid early drying of the leaf discs. Single third instar larva of the respective insects was introduced into each Petri dish. Progressive consumption of treated and control leaves by the larvae after 24 h was assessed using the Leaf Area Meter (Delta-T Devices, Serial No. 15736 F 96, UK). Leaf area eaten by larva in the treatment was corrected from the negative control. Five replicates were maintained for each treatment with 10 larvae per replicate (total n = 50). The experiment was conducted at laboratory conditions (27 ± 2°C) with 14:10 h photoperiod and 75 ± 5% relative humidity. Antifeedant activity was calculated according to the formula of Bentley et al (1984).

**Larvicidal activity**

The protocol for larvicidal activity was as mentioned in antifeedant activity. After 24 h of treatment, the larvae were continuously maintained on the untreated fresh cotton and castor leaves for *H. armigera* and *S. litura*, respectively. Diet was changed every 24 h. Larval mortality was recorded up to 96 h of treatment. Percent mortality was calculated according to Abbott's (1925) formula:

$$\text{Corrected mortality} = \frac{\text{\% mortality in treatment} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \times 100$$

**Larval and pupal durations**
The treated larvae, which survived were continuously fed with fresh leaves. The larval duration was calculated after treated larvae became pupae. Pupal duration was calculated from the day of the emergence of adults from pupae.

**Adult emergence**
The treated pupae were maintained until the emergence of adults. Adult emergence was calculated by the number of emerging adults from the total number of pupae.

**Mosquito larvicidal activity**
Bioassays were studied using the method of World Health Organization (1996) with second and fourth instar larvae of *An. stephensi* and *Cx. quinquefasciatus*. Flindersine was used at 2.5, 5, 10 and 20 ppm concentrations. Five replicate were maintained, each comprising 25 larvae (total N= 125). For the experiments, 249 mL dechlorinated tap water containing 0.1% Tween 80 and 1.0 mL of the required concentration of test solution were mixed.Tween80 was used as a negative control. Mortality rate was observed after 24 h of treatments.

**Statistical analysis**
All the data were analysed using one way ANOVA. Significant differences between the treatments were determined using DMRT tests ($P \leq 0.05$). Linear regression analysis was performed for all dose-response experimental data, and regression analysis was done between antifeedant and other biological activities. $LC_{50}$ and $LC_{90}$ values were calculated using probit analysis (Finney 1971).

## RESULTS

**Antifeedant activity**
Table 1 shows the antifeedant activity of flindersine against *H. armigera* and *S. litura*. Maximum antifeedant activity of 84.24 and 78.07% was observed at 1000 ppm concentrations against *H. armigera* and *S. litura*, respectively. All the tested concentrations showed more than 50% antifeedant activity against both the pests. Flindersine showed concentration dependent activity against both the pests. The regression ($R^2$) values of 0.91 and 0.87 were seen for *H. armigera* and *S. litura*. respectively. Flindersine and azadirachtin showed similar regression values ($R^2$) of 0.91 and 0.91 for *H. armigera* and $R^2$ 0.88 and 0.87 for *S. litura*, respectively. All the original data showed normality in Shapiro-Wilk test.

**Larvicidal activity**
Flindersine showed maximum larvicidal activity of 79.11 and 69.33% at 1000 ppm concentration against *H. armigera* and *S. litura*, respectively. At 125 ppm concentration, flindersine exhibited 24.88 and 22.44% larvicidal activity against *H. armigera* and *S. litura*, respectively. It exhibited good regression value ($R^2$) of 0.87 and 0.90 for *H. armigera* and *S. litura*, respectively. It exhibited $LC_{50}$ values of 443.04 and 566.88 ppm for larvicidal activity against *H. armigera* and *S. litura*, respectively (Table 2). Flindersine showed good regression value when compared to azadirachtin against both the pests. All the data did not show normality at Shapiro-Wilk test even after tansformation of data.

**Table 1 -** Percent antifeedant activity of flindersine against *Helicoverpa armigera* and *Spodoptera litura.*

| Compound | Concentration ppm | *H. armigera* | $R^2$ | Regression equation | *S. litura* | $R^2$ | Regression equation |
|---|---|---|---|---|---|---|---|
| Flindersine | 125 | 53.64±3.38$^{b}$ | 0.91 | y=51.43x+0.04 | 50.66±3.28$^{b}$ | 0.87 | y=49.99x+0.03 |
| | 250 | 60.39±1.55$^{c}$ | | | 59.39±5.27$^{c}$ | | |
| | 500 | 73.80±3.31$^{d}$ | | | 67.08±2.47$^{d}$ | | |
| | 1000 | 84.24±1.56$^{e}$ | | | 78.07±4.57$^{e}$ | | |
| Azadirachtin | 125 | 54.54±3.67$^{b}$ | 0.91 | y=51.10x+0.04 | 51.76±3.25$^{b}$ | 0.88 | y=50.91x+0.04 |
| | 250 | 62.43±3.38$^{c}$ | | | 64.03±5.05$^{cd}$ | | |
| | 500 | 74.13±2.82$^{d}$ | | | 73.89±5.21$^{e}$ | | |
| | 1000 | 88.94±5.02$^{f}$ | | | 89.64±4.49$^{f}$ | | |
| Control | | 3.02±1.39$^{a}$ | | | 2.09±1.12$^{a}$ | | |

Means followed by the same letter do not differ significantly by using DMRT ($P \leq 0.05$) and complete regression equation. The results represent the mean ± Standard Deviation.

**Table 2 -** Percent larvicidal activity and effective concentration of flindersine against *Helicoverpa armigera* and *Spodoptera litura*.

| Compound | Concentration ppm | *H. armigera* | $LC_{50}$ | $R^2$ | Regression equation | *S. litura* | $LC_{50}$ | $R^2$ | Regression equation |
|---|---|---|---|---|---|---|---|---|---|
| Flindersine | 125 | 24.88±4.75[a] | | | | 22.44±4.33[a] | | | |
| | 250 | 39.55±3.97[b] | 443.04 | 0.87 | y=24.17x +0.06 | 34.66±5.05[b] | 566.88 | 0.90 | y=20.68x +0.05 |
| | 500 | 62.44±5.79[d] | | | | 53.11±4.54[d] | | | |
| | 1000 | 77.11±4.12[e] | | | | 69.33±1.49[f] | | | |
| Azadirachtin | 125 | 48.88±4.64[c] | | | | 40.66±5.96[c] | | | |
| | 250 | 66.00±3.65[d] | 145.69 | 0.72 | y=52.14x +0.06 | 63.33±4.71[e] | 175.97 | 0.73 | y=45.43x +0.06 |
| | 500 | 97.77±4.96[f] | | | | 95.77±5.79[g] | | | |
| | 1000 | 100.00±0.00[f] | | | | 100.00±0.00[g] | | | |

Means followed by the same letter do not differ significantly by using DMRT (P≤ 0.05) and complete regression equations. The results represent the mean ± Standard Deviation.

## Growth inhibitory activity

Flindersine showed different kind of growth inhibitory activities against *H. armigera* and *S. litura*. It exhibited maximum larval duration of 14.66 and 15.10 days for *H. armigera* and *S. litura*, respectively at 1000 ppm concentration. At 125 ppm concentration, it also significantly increased the larval duration to 10.87 and 10.49 for *H. armigera* and *S. litura*, respectively, when compared to control (Table 3). Maximum pupal durations of 16.8 and 16.3 days were observed for *H. armigera* and *S. litura*, respectively at 500 ppm concentration (Table 3). When compared to control, flindersine at all the concentrations showed significant activity against both the pests. All the original larval-pupal duration data showed normality at Shapiro-Wilk test. No adult emergence was observed in *H. armigera* and *S. litura* at 1000 ppm concentration of flindersine and at 125 ppm, it showed 68.45 and 63.80% adult emergence in *H. armigera* and *S. litura* respectively. At 500 ppm, less than 50% of adult emergence was observed in both the pests. Increased concentration of flindersine reduced the adult emergence. Statistically significant activity (adult emergence) was observed in all the concentrations of flindersine when compared to control. Due to post injective effect of flindersine, the larvae consumed low amount of untreated diet.

**Table 3 -** Growth inhibitory activity of flindersine against *Helicoverpa armigera* and *Spodoptera litura.*

| Compound | Concentration ppm | *H. armigera* | | | *S. litura* | | |
|---|---|---|---|---|---|---|---|
| | | Larval duration days | Pupal duration days | Adult emergence | Larval duration days | Pupal duration days | Adult emergence |
| Flindersine | 125 | 10.87±0.57[b] | 12.17±0.32[b] | 68.45±4.83[d] | 10.49±0.22[b] | 11.9±0.28[b] | 63.80±6.38[d] |
| | 250 | 12.14±0.54[c] | 14.33±0.52[c] | 50.57±9.34[c] | 11.79±0.38[c] | 13.9±0.65[c] | 46.66±10.27[c] |
| | 500 | 13.21±1.03[de] | 16.8±0.83[d] | 30.00±4.56[b] | 13.01±0.21[d] | 16.3±0.67[d] | 36.00±3.65[b] |
| | 1000 | 14.66±0.77[f] | - | 00.00±0.00[a] | 15.10±0.41[f] | - | 00.00±0.00[a] |
| Azadirachtin | 125 | 12.61±0.97[cd] | 16.6±0.89[d] | 30.00±9.35[b] | 12.03±0.47[c] | 15.8±0.27[d] | 42.66±7.22[bc] |
| | 250 | 13.73±0.56[ef] | - | 00.00±0.00[a] | 14.23±0.22[e] | - | 00.00±0.00[a] |
| | 500 | - | - | - | - | - | - |
| | 1000 | - | - | - | - | - | - |
| Control | | 9.21±0.41[a] | 9.92±0.26[a] | 95.55±6.08[e] | 9.35±0.19[a] | 10.11±0.35[a] | 97.77±4.96[e] |

Means followed by the same letter do not differ significantly by using DMRT (P≤ 0.05). Values were Mean ± SD.

## Regression value

The regression value is an important factor. All the biological activities are directly dependent on the consumption of food. After 24 h of treatment, the larvae were unable to feed normally. The larval-pupal durations and adult emergence varied when compared to control due to post injective effects of flindersine. It showed good regression values between antifeedant and larvicidal activities ($R^2$ 0.94 and 0.87), antifeedant and larval duration ($R^2$ 0.79 & 0.89), antifeedant and pupal duration ($R^2$ 0.91 and 0.79) and antifeedant and adult emergence ($R^2$ 0.93 & 0.88) for *H. armigera* and *S. litura*, respectively (Table 4).

**Mosquito larvicidal activity**

In the present study, flindersine exhibited 100% larvicidal activity against *An. stephensi* and *Cx. quinquefasciatus* at 20 ppm concentration (Table 5). It manifested least $LC_{50}$ value of 1.68 ppm (95% confidential interval 1.11-2.14 ppm) against 2nd instar larvae of *An. Stephensi,* followed by 2.71 ppm (95% confidential interval 2.18-3.15 ppm) for 4th instar larvae of *An. stephensi*. In the case of *Cx. quinquefasciatus,* flindersine exhibited $LC_{50}$ value of 2.90 (95% confidential interval 2.38-3.35 ppm) and 4.19 (95% confidential interval 3.74-4.61 ppm) against 2nd and 4th instar larvae, respectively. All the tested concentrations exhibited more than 40% larvicidal activity against both vector mosquitoes. Toxicity was concentration dependent. At 10 ppm, flindersine exhibited more larvicidal activity against both the tested mosquitoes.

**Table 4 -** Linear regression relationship between biological activities on treated insects.

| Regression between | Insects | Treatment | R | $R^2$ | Regression equation | F | P |
|---|---|---|---|---|---|---|---|
| Antifeedant and larvicidal | *H. armigera* | Flindersin | 0.97 | 0.94 | y=38.33x+0.58 | 295.9 | 0.00 |
| | | Azadirachtin | 0.87 | 0.76 | y=28.30x+0.53 | 56.4 | 0.00 |
| | *S. litura* | Flindersin | 0.93 | 0.87 | y=39.38x+0.54 | 122.9 | 0.00 |
| | | Azadirachtin | 0.89 | 0.78 | y=31.06x+0.52 | 64.8 | 0.00 |
| Antifeedant and larval duration | *H. armigera* | Flindersin | 0.89 | 0.79 | y= -21.44x+7.01 | 65.8 | 0.00 |
| | | Azadirachtin | 0.43 | 0.19 | y=26.68x+2.42 | 1.87 | 0.21 |
| | *S. litura* | Flindersin | 0.94 | 0.89 | y=-8.9x+5.8 | 129.4 | 0.00 |
| | | Azadirachtin | 0.80 | 0.63 | y=-7.8x+4.9 | 13.73 | 0.00 |
| Antifeedant and pupal duration | *H. armigera* | Flindersin | 0.95 | 0.91 | y=-0.18x+4.3 | 126.3 | 0.00 |
| | | Azadirachtin | 0.76 | 0.57 | y=106.2x+-3.1 | 4.01 | 0.14 |
| | *S. litura* | Flindersin | 0.90 | 0.79 | y=10.37x+3.5 | 49.13 | 0.00 |
| | | Azadirachtin | 0.41 | 0.21 | y=-68.01x+7.6 | 2.01 | 0.25 |
| Antifeedant and Adult emergence | *H. armigera* | Flindersin | 0.94 | 0.93 | y=84.82x+-0.46 | 259.8 | 0.00 |
| | | Azadirachtin | 0.73 | 0.53 | y=61.92x+-0.23 | 9.1 | 0.02 |
| | *S. litura* | Flindersin | 0.94 | 0.88 | y=78.89x+-0.41 | 129.3 | 0.00 |
| | | Azadirachtin | 0.87 | 0.74 | y=64.01x+-0.29 | 23.88 | 0.00 |

* significant at ($P \leq 0.05$) level. Values were Mean ± SD.

**Table 5 -** Larvicidal activity of Flindersine against vector mosquitoes of *Anopheles stephensi* and *Culex quinquefasciatus.*

| Instars | Concentration (ppm) | % larvicidal activity | $LC_{50}$ | 95% confidential interval | | $LC_{99}$ | 95% confidential interval | | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | Lower | Upper | |
| | | | | *Cx. quinquefasciatus* | | | | | |
| 2nd | 2.5 | 48.73±3.57 | 2.90 | 2.38 | 3.35 | 15.76 | 14.52 | 17.34 | 16.20 |
| | 5 | 62.74±4.51 | | | | | | | |
| | 10 | 90.19±4.40 | | | | | | | |
| | 20 | 100.0±0.00 | | | | | | | |
| 4th | 2.5 | 40.45±3.68 | 4.19 | 3.74 | 4.61 | 18.21 | 16.89 | 19.54 | 13.41 |
| | 5 | 54.53±4.58 | | | | | | | |
| | 10 | 81.77±3.81 | | | | | | | |
| | 20 | 100.0±0.00 | | | | | | | |
| | | | | *An. stephensi* | | | | | |
| 2nd | 2.5 | 58.34±2.11 | 1.68 | 1.11 | 2.14 | 11.76 | 10.79 | 13.04 | 26.15 |
| | 5 | 76.63±4.70 | | | | | | | |
| | 10 | 97.53±3.61 | | | | | | | |
| | 20 | 100.0±0.00 | | | | | | | |
| 4th | 2.5 | 47.42±3.14 | 2.71 | 2.18 | 3.15 | 15.04 | 13.86 | 16.55 | 12.21 |
| | 5 | 68.39±4.06 | | | | | | | |
| | 10 | 90.85±3.47 | | | | | | | |
| | 20 | 100.0±0.00 | | | | | | | |

Values were Mean ± SD.

## DISCUSSION

### Antifeedant activity

Flindersine isolated from *T. asiatica* was studied for biological activities against *H. armigera* and *S. litura*. Generally, alkaloid compounds act as a stomach poison. In the present study, flindersine showed maximum antifeedant activity of 84 and 78% against *H. armigera* and *S. litura*, respectively at 1000 ppm concentration, which confirmed the findings of Cornelius et al. (2009) who reported that erythrina alkaloids of (+)-11β-methoxy-10-oxoerysotramidine from *Erythrina latissima* (Fabaceae) showed 80.9% antifeedant activity against *S. littoralis* Boisduval at 1000 ppm concentration. Furoquinoline alkaloids (dictamnine and evolitrine) derived from *Evodia lunuankenda* (Rutaceae) showed antifeedant activity against *S. litura* (Jagadeesh et al. 2000). The present result was also supported by the findings of Il-Kwon et al (2000) who reported that isoquinoline alkaloids of palmatine iodide and berberine chloride from *Coptis japonica* (Ranunculaceae) showed more than 75% antifeedant activity against *Hyphantria cunea* Drury. Dihydro-β-agarofuran, derived from *Parnassia wightiana* (Celastraceae) showed high antifeedant activity against *Mythimna separata* Walker (Jiang-Jiang et al. 2013).

### Larvicidal activity

In this study, flindersine showed $LC_{50}$ values of 443.04 and 566.88 ppm against *H. armigera* and *S. litura*, respectively. Similarly, Emam et al. (2009) reported that furocoumarin alkaloid- 3(2",2"dimethyl butenyl) 3′ - hydroxy dihydrofuropsoralen and quinolone alkaloid-rutamine exhibited larvicidal activity against *S. littoralis* with $LC_{50}$ values of 1598 and 1215 ppm, respectively. Pipernonaline and piperoctadecalidine isolated from *Piper longum* (Piperaceae) showed insecticidal activity with $LC_{50}$ values of 530 and 235 ppm, respectively against *Myzus persicae* Sulzer and also showed insecticidal activity against *Nilaparvata lugens* (Stål) and *S. litura* (Park et al. 2002). Moreira et al. (2007) reported that 5,6,7,8,3′,4′, 5′-heptamethoxyflavone and 5,6,7,8,3′-pentamethoxy-4′,5′-methylenedioxyflavone from *Ageratum conyzoides* (Asteraceae) showed insecticidal activity against *Rhyzopertha dominica* Fabricius, *Diaphania hyalinata* Linnaeus, *Musca domestica* Linnaeus and *Periplaneta americana* Linnaeus.

### Growth inhibition

Flindersine showed growth inhibitory activities such as increased larval-pupal duration and reduction in adult emergence in *H. armigera* and *S. litura.* The present finding corroborated with those of Acheuk and Doumandji-Mitiche (2013) who reported that the extracts containing alkaloid from *Pergularia tomentosa* (Apocynaceae) affected the larval duration and adult emergence of *Locusta migratoria cinerascens* Fabricius; the larval duration increased up to 18.0 days after the treatments when compared to control and adult emergence significantly decreased. Hexane extracts and their fractions containing alkaloids from *Atalantia monophylla* (Rutaceae) completely prevented the adult emergence of *H. armigera* (Baskar et al. 2009). In the present study, larval-pupal duration increased and adult emergence was reduced. These findings were in agreement with Sakr et al. (2013) who reported that 5-hydroxy-7,4'-dimethoxy-flavon-3-ol and 5-hydroxy-7-methoxy-2-(4`-methoxy-phenyl)-chromen-4-one isolated from a dichloromethane extract of *Hyptis brevipes* (Lamiaceae) showed growth inhibitory activity against *S. littoralis*. Similarly, many workers have reported that naturally purified, semi purified compounds and extracts exhibited growth inhibitory activity against agricultural pests. Baskar and Ignacimuthu (2012) reported that ononitol monohydrate from *Cassia tora* (Fabaceae) increased the larval-pupal durations and inhibited the adult emergence of *H. armigera* and *S. litura*. Hexane and ethyl acetate extracts from the leaf and root of *Aristalochia tagala* (Aristolochiaceae) increased the larval-pupal duration and reduced the adult emergence (Baskar et al. 2011). In the present study, flindersine exhibited no adult emergence at higher concentration in *H. armigera* and *S. litura*. These findings supported the earlier findings of Munoz et al. (2013) who reported that hexane, ethyl acetate and methanol extracts from *C. talcana* reduced the adult emergence of *S. frugiperda*.

### Regression value

Flindersine showed high regression value between antifeedant and other parameters in this study. Flindersine showed good regression value (R 0.94) between antifeedant and adult emergence for the two tested insects. These finding corroborated with

Gonzalez-Coloma et al. (1995) reported that 11β-acetoxy-5-angeloyloxy-silphinen-3-one from *Senecio palmensis* (Asteraceae) showed good regression value (R 0.82) between relative consumption and relative growth rates in *Leptinotarsa decemlineata* Say. Flindersine showed good regression value between the concentration and antifeedant activity of the tested insects. Similarly, Wheeler and Isman (2001) reported that *Trichilia americana* (Meliaceae) extracts showed high regression value ($R^2$ 0.96) between relative consumption and relative growth rate in *S. litura*. Osthol [2H-1-Benzopyran-2-one, 7-methoxy-8-(3-methyl-2-butenyl) from chloroform root extract of *Clausena anisata* (Rutaceae) showed high regression ($R^2$ 0.82) between concentration and antifeedant activity in *H. armigera* (Pitan et al. 2009). In this study, flindersine showed more than 0.80 R value between antifeedant and larvicidal activity in *H. armigera* and *S. litura*. These findings corroborated with the findings of Baskar et al. (2014) who reported that friedelin showed more than 0.80 R value between antifeedant and larvicidal activity against *H. armigera* and *S. litura*.

**Mosquito larvicidal activity**

In the present study, flindersine exhibited 100% larvicidal activity with less than 5 ppm $LC_{50}$ against *An. stephensi* and *Cx. quinquefasciatus*. This result corroborated with the findings of Maheswaran and Ignacimuthu (2013) who reported that confertifolin from *Polygonum hydropiper* (Polygonaceae) exhibited less than 5 ppm $LC_{50}$ against *An. stephensi* and *Cx. quinquefasciatus*. Pectolinaringenin from *Clerodendrum phlomidis* (Lamiaceae) exhibited larvicidal activity against *Cx. quinquefasciatus* and *A. aegypti* (Muthu et al. 2012). Similarly, essential oil and their compounds sabinene, biofloratriene, borneol and β-Bisabolol from *Clausena dentate* (Rutaceae) exhibited larvicidal activity against *A. aegypti* (Rajkumar and Jebanesan 2010). Lima et al. (2014) reported that *Mentha* x *villosa* (Lamiaceae) essential oil showed larvicidal activity against *A. aegypti*. Root extract of *Tragia involucrate* (Euphorbiaceae) exhibited more than 90% larvicidal activity against *Cx. quinquefasciatus* **(**Bhattacharya and Chandra 2014).

## CONCLUSION

In the present study flindersine, an alkaloid showed significant antifeedant, larvicidal, growth inhibitory activities against *H. armigera* and *S. litrua*. It showed 100% larvicidal activity against vector mosquitoes at 20 ppm concentration. Larvicidal is main methods to control and manage the vector mosquitoes. Antifeedancy is a primary factor, which influences larval death, larval-pupal duration and adult emergence of the pests. High correlation between antifeedant and larvicidal and growth inhibitory values were recorded. Flindersine could be a suitable compound to develop a new botanical pesticide formulation to control vector mosquitoes and agricultural pests.

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