International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 04-02-2021, Accepted: 08-03-2021, Published: 14-04-2021
Volume 6, Issue 2, 2021, Page No. 228-234

Larvicidal activity of ethanolic leaf extract of *Tylophora indica* (Burm.f.) on *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae)

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Abstract
Rice moth *Corcyra cephalonica* is a major pest of stored cereals and other commodities in India as well as in other tropical and subtropical regions of the world. Different concentrations of *Tylophora indica* leaves extracts were mixed flour and *C. cephalonica* larva were reared on the mixed flour and the mortality was recorded. LD_{50} values recorded for the fifth instar *C. cephalonica* exposed to *T. indica* for 36 hours was 3.502 mg/25 g and the corresponding values recorded for 96 hours was 2.164 mg/ 25 g. Phytochemical analysis of *T. indica* leaves extracts revealed the presence of cynogenic glycosides, flavanoides, tannins and phenols. The GC-MS result clearly indicated the presence of certain bioactive compounds like Squalene, 1, 2 Benzenedicarboxlicacid disooctyl ester. Since the plant had insecticidal properties they are potentially suitable for integrated pest management.

Keywords: larvicidal activity, ethanolic extract, phytochemicals, gc-ms

Introduction

In the recent years, the use of synthetic pesticides for pest management has become highly controversial. The indiscriminate use of chemical pesticides has given rise to many serious costs of applications and environmental pollution hazards from handling. However, insecticides have serious disadvantages such as pest revival and resistance lethal effects on non-target organisms, the risk of user's contamination food scraps and environmental pollution (Tapondjou *et al*., 2002) [38]..

The rice moth, *Corcyra cephalonica* [Stainton] (Lepidoptera: Pyralildae) is the major and important pests of stored commodities in the tropics (Lucas and Riudavets, 2002) [22]. Asia, South America and Africa (Allotey and Azalekor, 2000 [2]. Huang and Subramanyam, 2004) [15]. The *Corcyra cephalonica* [Stainton] larvae feed on rice, corn, cocoa, chocolate, dried fruit, biscuits, coffee and other seeds (Rao, 1954 and Usda, 1986) [31, 40]. The rice moth is a worldwide pest of stored foodstuffs. Control of these insects generally requires the use of chemical insecticides that are toxic to humans and domestic animals and harmful to the environment (Coelho *et al*., 2007) [7].

In addition the larvae while feeding, leaving silken threads and contaminate the grain by producing dense webbing containing their fecal material and cast skins. The webbing formed is detectable thick and tough adding the damage caused (Allotey and Azalekor, 2000) [2]. For the control of stored produced insects, it is regularly safer to use plant materials with insecticidal, antifeedant or repellent properties than the use synthetic insecticides (Prakash and Rao, 1997; Allotey and Azalekor, 2000) [30, 2].

Several stored products insects have been reported to be resistant to pirimiohosmethyl (Perez, 1999) [29]. Different plant products have been used to control *C. cephalonica*, it has been reported that B-N-Oxalyl-Lαβ-diaminopropionic acid (L-BoAAd) isolated from the plant *Lathyrus satius* has an inhibitory effect on the growth of rice moth, *C. cephalonica* larvae (Diallo *et al*., 2001) [8].

Many plant extracts possess cost-effective and ecofriendly molecules that can be effectively used as mosquito repellents and pesticides without any side effects to non-target organisms.

The active molecules are reported from different parts of plants namely fruit pulp, kernel, root, bark, and leaf (Ansari *et al*., 2000; Ezeonu *et al*., 2001; Sen-Sung *et al*., 2003; Chapagain *et al*., 2007; Rawani *et al*., 2013). Kani *et al*. (2012, 2012a) [3, 9, 35, 5, 32, 17, 18]. Studied the bioactive effect of plant extracts *Piper nigrum* and *Jatropha curcas* extracts against rice pest *Corcyra cephalonica*.

Both plant extracts had high bioactivity against *C. cephalonica* larvae and antifeedant action was increased with increasing plant extract concentrations. Khan and Qamar (2015) [19]. Conducted a study to evaluate the antifeedant and larvicidal activity of commercial pesticides and plant extracts on rice moth C. *cephalonica*.

They concluded plant extracts have feeding deterrent and toxic effects which are compared favourable to the commercial biopesticides. *Azadirachta indica* and Eucalyptus globules leaves powder have inseciticidal effect against rice moth *C. cephaloica* (Singh *et al*., 2019) [37].

Tylophora indica (Burm f.) Merill. (Syn: Tylophora asthmatica; Asclepias asthmatica), a twining perennial woody plant of family Asclepidaceae, is widely distributed in Africa, Asia, Australia, and Oceanic islands (Nema *et al*., 2007) [27].

Tylophora indica has been reported to contain 0.2- 0.46% alkaloids *viz*. tylophorine, tylophorinine, tylophorinidine, (+) septicine, isotylocrebrine, tylophorinicine, sterols, flavanoids, wax, resins, and tannins (Govindhari *et al*., 1975) [12]. Shahzad *et al*., 2016) [36].The plant have many medicinal properties such as antitumor (Saraswathi *et al*.,

2013) [34]. Antioxidant (Bhatia *et al*., 2013) [4]. Antirehumatic (Reddy *et al*., 2009) [33]. Antiasthmatic (Mohiuddin, 2019 [26]. Umamaheswari *et al*., 2017) [39]. Hepatoprotective (Manikkoth and Rao, 2013) [24]. Anti-neroinflammatory (Gupta *et al*., 2020) [13]. And antifeedant activities (Reddy *et al*., 2009) [33]. Although, the leaves extract of *T. indica* has larvicidal and repellent activities against mosquitoes *Culex quinquefasciatus* and *Aedes aegypti* (Gandhi *et al*., 2014) [11]. The present study was aimed to evaluate the larvicidal activity of ethanolic leaf extract of *Tylophora indica* against *Corcyra cephalonica*.

Materials and methods

Plant materials

Fresh leaves of *Tylophora indica* Burm.f. Were collected from Scott Christian College, campus and brought to the laboratory for extraction. Only the fresh leaves were collected leaving behind the very tender and old ones. All the leaves were washed thoroughly with distilled water. The leaves were shade dried, after which the leaves were powdered with the aid of an electric blender and stored in a tightly closed container for future use.

Preparation of extracts

The powdered material (15 g) was packed into a thimble made of Whatman filter paper No.1. Crude ethanolic extract was obtained using soxhlet extraction apparatus by adding 15 g of powdered material into 200 ml of ethanol solvent for 48 hours. The extract was evaporated to yield crude ethanolic extract.

Procurement and maintenance of the test species

The eggs of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) obtained from the Tamil Nadu Agriculture University (TANU), Coimbatore, were allowed to hatch in petriplates containing wheat flour. The hatched out caterpillars were transferred to petriplates containg mixture of wheat, maize and other milled flours. The growth of the caterpillars were followed and the adult moths were allowed to lay eggs. The eggs were used for further studies.

Toxicity Bioassay

The newly emerged fifth instar larvae of *C. cephalonica* (Stainton) were introduced into separate containers containing 25 g milled flour and different concentration of *Tylophora indica* (Burm.f.) (Range 0.5 to 5.0 mg stock/25 g flour). Three replicates were maintained for each concentration. Each set was maintained by regularly topping up with the experimental feed and the mortality were recorded after different hours of exposure and the data were subjected to probit analysis (Finney, 1971) [10]. LD_{50} values and their upper and lower confidence intervals were calculated.

Phytochemical analysis

The extracts were subjected to various phytochemical tests to determine the active constituents present in the ethanolic extracts. Tests were performed for cynogenic glycosides, flavonoids, terpenoids, tannins, saponins and polyphenols by standard methods (Harborne, 1973 and Obadoni and Ochuko, 2001) [14, 28].

GC-MS analysis

The bioactive compounds present in the leaf extract was analysed using GC-MS (THERMO GC- TRACE ULTRA VER: 5.0, THERMO MS DOSQ 11).

Results

The phytochemical analysis of ethanolic extract of *T. indica* showed the presence of cynogenic glycosides, flavonoids, tannins, phenols whereas terpenoids and saponins were absent (Table 1).The plant *T. indica* used for extract and *C. cephalonica* were showed in figure 2.

Table 1: Phytochemical analysis of Tylophora indica

Phytochemicals analyzed	*Tylophora indica*
Cyanogenic glycosides	+
Flavonoids	+
Terpenoids	-
Tannins	+
Saponins	-
Phenols	+

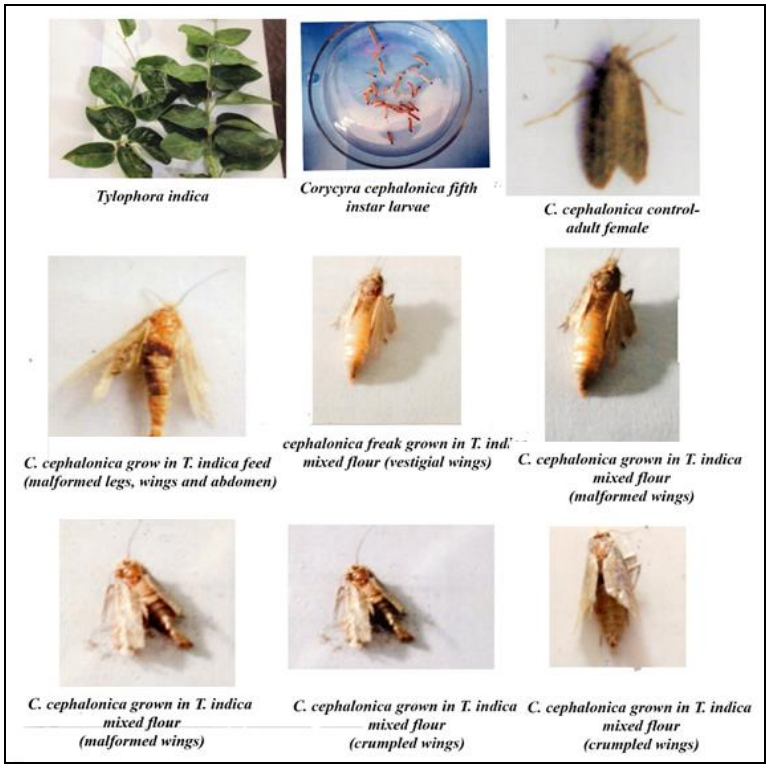

Fig 1: T. indica and Corcyra cephalonica adults showing malformed body parts

The GC-MS pattern of the ethanolic extract of *T. indica* recorded 8 major peaks with the retention time 5.60, 10.09, 16.79, 19.80, 24.02, 26.35, 34.48 and 38.28 minutes. The possible compounds recorded for the different peaks were analysed by mass spectrum. The possible compounds recorded for the peak1 (RT: 10.09 minutes) is cycloheptasiloxane tetradecamethyl, tetradecamethyl cycloheptasiloxane. The possible compounds of Guanosine (CAS), a-D-Glucopyranoside, a-D-fructofuranosyl were recorded for the peak 2 (RT: 16.79 minutes). The analysis of 3rd peak hows different compounds like 2-Hexadocon-1-ol, 3, 7, 11, 15-tetramethyl- [R-[R*, R*-(E)]] – [CAS], Neophytadiene, (RT: 1980 minutes). Phthalic acid butylundecyleser, Phthalic acid isobutyl pentadecyl ester were identified in the 4th peak (R.T.24.02 minutes) and 2-Hexadecen-1-ol, 3, 7, 11, 15 - tetramethyl -[R-[R*, R*-(E)]] – [CAS], phytol isomer were observed for 5th peak. (RT: 26.35 minutes). The possible compounds of 1, 2-Benzenedicarbacylic acid bis (2 ethylhexyl) ester (CAS), 1, 2-Benzenedicarboxylic acid diiscooctyl eser were recorded for Th peak 6 (T: 34.48 minutes). The possible compounds recorded for the peak 7 (RT: 38.28 minutes) is 2, 6, 10, 14, 18, 22- Tetracosahexaene, 26, 10, 15, 19, 23- hexamethyl – (all-E) (Table 2 and Figure 2).

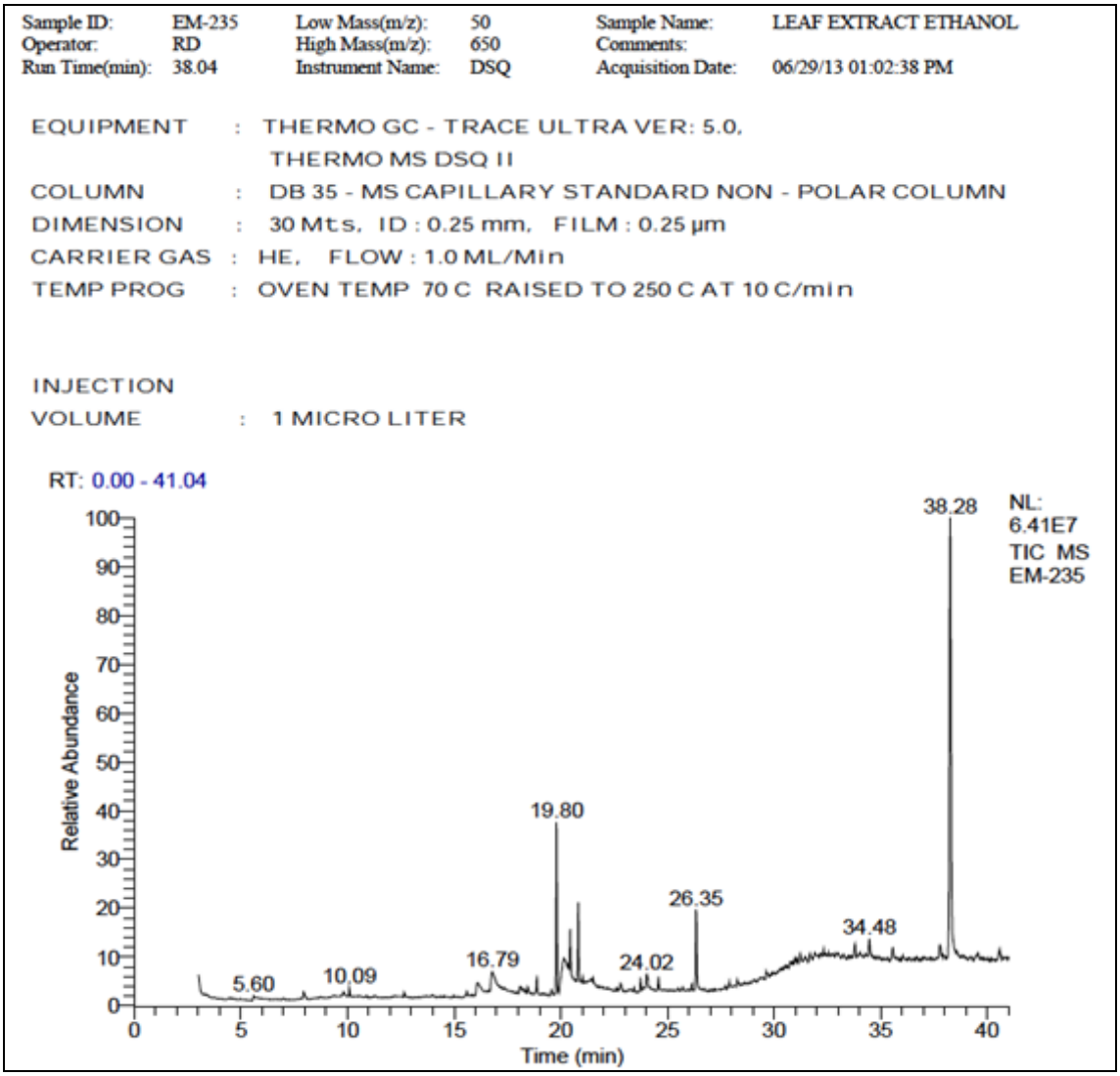

Fig 2: GC-MS Pattern of *T. indica* leaf extract

Table 2: Possible Compounds of Different Peak Observed in the GC-MS Pattern of *T. Indica* Leaf Extract

Retention Time	Possible Compounds	Strength Index (SI)	Relative Strength Index (RSI)	Molecular Formula	Molecular Weight
10.09	1. Cycloheptasiloxane, tetradecamethyl	820	899	$C_{14}H_{42}O_7Si_7$	518
	2. Tetradecamethylcyclo Heptasiloxane	820	898	$C_{14}H_{42}O_7Si_7$	518
	3. Isopropxy-1,1,1,7,7,7- hexamethyl – -3, 5, 5 – tris (trimethylsiloxy) tetrasiloxane	631	815	$C_{18}H_{52}O_7Si_7$	576
	4. c[si] (c) (c) o [si] (c) (c)(c) (o[si] (c) (c) (c) o[si] (o[si] (c) (c) (c) o[si] (TxT = Auto JL# 1- neat)	604	650	$C_{18}H_{54}O_7Si_8$	609
16.79	1. Guanosine (CAS)	678	681	$C_{10}H_{13}N5O5$	283
	2. Cytidine (CAS)	640	649	$C_9H_{13}N5O5$	243
	3. a-D-Glucopyranoside, a-D-Fructoturanosyl (CAS)	703	823	$C_{12}H_{22}O_{11}$	342
	4. 1-Sorbose	638	674	$C_6H_{12}O_6$	180

19.80	1. 2 – Hexadecen -1 –ol, 3, 7, 11, 15 – tetramethyl-, [R – [R*, R* - (E)]]-(CAS) 2. Neophytadiene 3. 3- Eicoyne 4. 3, 7, 11, 15- Tetramethyl – 2 – hexadecen – 1- ol	887	890	$C_{20}H_{40}O$	296
		882	886	$C_{20}H_{38}$	278
		799	802	$C_{20}H_{38}$	278
		879	903	$C_{20}H_{40}O$	296
24.02	1. Phthalic acid, butyl undecyl ester 2. Phthalic acid, isobuthyl pentadecyl ester 3. Phthalic acid, isobutyl octadecyl ester 4. Phthalic acid, decyl isobutyl ester	674	859	$C_{23}H_{36}O_4$	376
		670	864	$C_{27}H_{44}O_4$	432
		654	855	$C_{30}H_{50}O_4$	474
		667	879	$C_{22}H_{34}O_4$	362
26.35	1. 2- Hexadecen – 1 – ol, 3, 7, 11, 15 – tetramethyl -, [R – [R* R* - (E)]]-(CAS) 2. Phytol Isomer 3. Phytol	873	883	$C_{20}H_{40}O$	296
		882	893	$C_{20}H_{40}O$	296
		856	863	$C_{20}H_{40}O$	296
34.48	1. 1, 2 – Benzenedicarboxylic acid, bis (2ethylhexyl) ester (CAS) 2. 1, 2 – Benzenedicarboxylic acid, diisooctyl easter 3. 1, 2 – Benzenedicarboxylic acid 4. 1, 2 – Benzenedicarboxylic acid, mono (2 – ethyl hexyl) ester	681	826	$C_{24}H_{38}O_4$	390
		676	853	$C_{24}H_{38}O_4$	390
		676	853	$C_{24}H_{38}O_4$	390
		667	845	$C_{16}H_{22}O_4$	278
38.28	1. 2, 6, 10, 14, 18, 22 – Tetracosahexaene, 2, 6, 10, 15, 19, 23 – hexamethyl -, (all-E), 2. Squalene 3. 2, 6, 10, 14, 18 – Pentamethyl – 2, 6, 10, 14, 18 – eicosapentaene	950	951	$C_{30}H_{50}$	410
		873	874	$C_{30}H_{50}$	410
		846	855	$C_{25}H_{42}$	342

The fifth instar *C. cephalonica* larvae were exposed to 10 different concentrations of *T. indica* leaf powder ranging from 0.5 to 5.0 mg concentration. At 1.5 mg concentration of *T. indica* extract 20% mortality was observed after 96 hours of exposure. All the exposed larvae died (100% mortality) after 96 hours in 3.5 mg concentration. In the fifth instar the mortality was 10% after 60 hours of exposure to 2.0 mg *T. indica* and 70% after 120 hours. At 4.5 mg concentration of *T. indica* extract 100% mortality was observed after 48 hours of exposure. All the exposed larvae died (100% mortality) after 24 hour in 5.0 mg concentration (Figure 3).

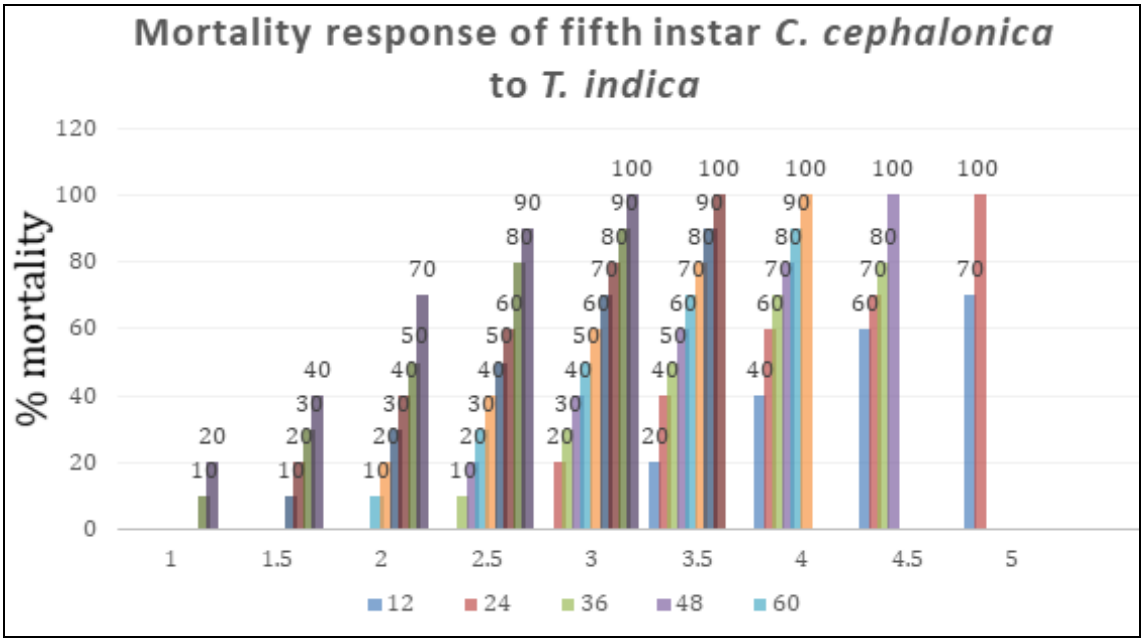

Fig 3: Mortality response of fifth instar *C. cephalonica* to *T. indica*

In 12 hours exposure of *C. cephalonica* to *T. indica* extract recorded the LD_{50} of 4.295, LCL 3.862 and UCL 4.773 mg respectively. Corresponding values for 24 hours was 3.744, 3.393 and 4.127 mg respectively. After 36 hours exposure the LD_{50} was 3.502, LCL 3.147 and UCL value was 3.893. In 48 hours exposure of *C. cephalonica* to *T. indica* extract the LD_{50} was 3.188, LCL 2.872 and UCL 3.537 mg respectively. Corresponding values for 60 hours was 2.931, 2.621 and UCL value was 3.031 mg. In 84 hours exposure of *C. cephalonica* to *T. indica* extract the LD_{50} was 2.418, LCL 2.105 and UCL 2.775 mg respectively. Corresponding values for 96 hours was 2.164, 1.857 and 2.521 mg respectively. After 108 hours exposure the LD_{50} was 1.838, LCL 1.551 and UCL value was 2.176 mg respectively. In 120 hours exposure of *C. cephalonica* to *T. indica* extract the LD_{50} was 1.551, LCL 1.291 and UCL 1.861 mg respectively.

Probit analysis of the 12 hours response of fifth instar *C. cephalonica* larvae recorded X of 1.627, Y of 4.945, b value of 8.928 mg and the corresponding value recorded for 24 hours exposure were 1.581, 5.101 and 12.555 mg. When the fifth instar larvae were exposed to *T. indica* extract for 36

hours the X of 1.542, Y of 4.978 and b value of 8.350 mg was recorded. Probit analysis of the 48 hours response of fifth instar larvae recorded a X of 1.516, Y of 5.141and b value of 11.404 mg The fifth instar larvae when exposed to *T. indica* extract for 60 hours the X value of 1.470, Y of 5.026 and b value 8.157 mg was recorded and the corresponding value recorded for 72 hours exposure were 1.443, 5.141 and 9.629 mg respectively. The 84 hours exposure of fifth instar larvae to *T. indica* recorded the X of 1.389, Y of 5.037 and b value 6.660 mg and the corresponding value recorded for 96 hours exposure were 1.353, 5.141 and 7.826 mg. In 108 hours exposure *C. cephalonica* larvae recorded a X of 1.279, Y of 5.081 and b value of 5.403 mg and the corresponding value recorded for 120 hours exposure were 1.228, 5.244 and 6.422 mg respectively with variance and chi square were tabulated in table 3.

Table 3: Record of an hour's lethality levels T. indica leaf extract to fifth instar C. cephalonica

S. No	n Hours	Regression equation	Lower limit	LC50	Upper limit	Variance	Chi-square
1	12	y=8.928x-9.58	3.862	4.295	4.773	0.0006	0.08
2	24	y=12.555x-14.75	3.393	3.744	4.127	0.0005	2.31
3	36	y=8.350x-7.89	3.147	3.502	3.893	0.0006	0.06
4	48	y=11.404x-12.5	2.872	3.188	3.537	0.0005	1.33
5	60	y=8.157x-6.97	2.621	2.931	3.277	0.0006	0.24
6	72	y=9.629x-8.75	2.621	2.679	3.031	0.0008	0.43
7	84	y=6.660x-4.21	2.105	2.418	2.775	0.0009	0.31
8	96	y=7.826x-5.45	1.857	2.164	2.521	0.0011	1.59
9	108	y=5.403x-1.83	1.551	1.838	2.176	0.0014	1.46
10	120	y=6.422x-2.65	1.291	1.551	1.861	0.0016	1.07

Discussion

Preliminary phytochemical screening of ethanolic extract of T. indica Burm.f. Revealed the presence of cynogenic glycosides, tannins, phenols and flavonoids whereas the terpenods and saponins were absent in the leaf extract of *T. indica* Burm.f. These results were supported by Maheshwari and Vijayarangan (2020), Mohan *et al*. (2014) and Kumar *et al*. (2011). These phytochemicals may be responsible for the insecticidal properties (Kabaru and Gichia, 2001). [23, 25, 20, 6, 16]. GC-MS analysis of *T. indica* Burm.f. Leaf extract showed the presence of squalene at the retention time of 38.28 minutes, which was supported by Kumari *et al*., 2012 [21]. Who identified similar compound was recorded in the leaf extract of *Sarcostemma secamone* L. with a retention time of 25.13 minutes. Maheshwari and Vijayarengan identified 18 compounds in the methanolic extract of *T. indica* leaves used by GC-MS. Although, Abirami and Rajendran (2013) [1]. Identified the compound 1, 2-Benzenedicarboxylic acid, disooctyl ester with a retention time of 24.81 minutes in the leaf extract of *Vernonia cinaea.* The present investigation showed that different dose levels of *Tylophora indica* Burn.f. Leaf extract exerted a depressive effect on leaves of *C. cephalonica*. The results of this study indicated that the different concentration of *T. indica* significantly increased the mortality of 5th instar larvae of *C. cephalonica*. In the present study LD_{50} value recorded for 5th instar *C. cephalonica* fed with *T. indica* at 24 exposure was 3.744 mg. Chauhan *et al*. (2011) [6]. Reported that the extract from *Ocimum canum* Sims. *Ocimum sanctum* L. and *Rhinacanthus nasutus* L. with larvicidal activity and after 24 hours and showed that the methanolic extracts of *O. canum* Sims. And *R. naustus* L. with larvicidal activity (LC_{50} = 36.46 and 68.84 ppm respectively). The toxicity of *T. indica* leaf extract to fifth larvae of *C. cephalonica* increased with increase in concentration of the leaf extract. At 2.5 mg concentration of *T. indica* extract 100% mortality was observed after 96 hours of exposure. Ageratum conyzoides and Lantana camera showed good insecticidal activity against mustard aphids (*Lipaphis erysimi*) with percent mortality values ranging from 22.16 to 29.96% in Brassica (Srivastava and Guleria, 2003). The LD_{50} value recorded for fifth instar *C. cephalonica* exposed to T. indica extract was 2.164 mg for 96 hours of exposure. The leaf extract of *C. fistula* Linn. With different solvents like methanol, benzene and acetone were studied for the larvicidal and repellent activity against *A. aegypti* Linn. The 24 hours LC_{50} concentration of the extract against *A. aegypti* Linn. Were observed to be 10.69, 18.27 and 23.95 mg respectively for the three solvents. Which supported the present work (Govindarajan, 2009).

Conclusion

Results from the present study revealed that the extract of *T. indica* Burm.f. Possesses bioactive compounds which have been found to have a reasonable efficacy against the *Corcyra Cephalonia*, the biocide effect of plant may be due to the presence of active compounds such as tannin and phenol. Hence this extract can be used as natural source of biocide and isolation of specific constituents give better effect.

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