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Original Article

Efficacy of *Citrullus colocynthis* seed extract on *Earias vittella*, Fabricius, (Lepidoptera: Noctuidae): environment sustainable approach

Eficácia do extrato de semente de *Citrullus colocynthis* em *Earias vittella* Fabricius (Lepidoptera: Noctuidae): abordagem ambiental sustentável

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Abstract

Earias vittella Fabricius, 1794 (Noctuidae: Lepidoptera) is deliberated to be one of the most destructive pests of cotton and okra vegetation in the world including Asia. The pest has established resistance to various synthetic insecticides. The use of bio-pesticide is one of the unconventional approaches to develop a vigorous ecosystem without harming non- target pests and beneficial natural insect fauna. In the present study, the toxicity levels of *Citrullus colocynthis* seed extract have been evaluated against the populations of *E. vittella* under standardized laboratory conditions. The toxic effects of *C. colocynthis* on development periods, protein contents and esterase activity of the life stages of *E. vittella* were also evaluated. The toxicity levels of methanol, ethanol, hexane, water and profenofos were evaluated on the 1[st] instar larvae of *E. vittella.* LC_{30} and LC_{80} concentrations exhibited the effectiveness of methanol-based *C. colocynthis* seed extract against 1st instar larvae of *E. vitella.* The enhanced larval and pupal periods were revealed in treated samples during the comparison with untreated samples. The intrinsic rate of increase, net reproductive rate in the LC_{30} and LC_{80} concentrations exposed larvae remained less than the control treatment. Fecundity, the esterase activity and protein contents were declined in LC_{30} and LC_{80} treated samples as compared to the control. The present findings suggest that *C. colosynthis* extracts based botanical insecticides are beneficial, ecosystem sustainable and can be integrated with insect management programs from environment safety perspective.

Keywords: environment sustainable, plant extracts, ecosystem safety, esterase activity, life table parameters.

Resumo

Earias vittella Fabricius, 1794 (Noctuidae: Lepidoptera) é considerada uma das pragas mais destrutivas de algodão e quiabo no mundo, incluindo a Ásia. Essa praga estabeleceu resistência a vários inseticidas sintéticos. O uso de biopesticidas é uma das abordagens não convencionais para desenvolver um ecossistema saudável sem prejudicar as pragas não alvo e a fauna natural benéfica de insetos. No presente estudo, os níveis de toxicidade do extrato de semente de *Citrullus colocynthis* foram avaliados nas populações de *E. vittella* em condições de laboratório padronizadas. Os efeitos tóxicos de *C. colocynthis* nos períodos de desenvolvimento, conteúdo de proteína e atividade esterase das fases de vida de *E. vittella* também foram avaliados. Os níveis de toxicidade de metanol, etanol, hexano, água e profenofós foram avaliados em larvas de 1º instar de *E. vittella*. As concentrações de LC_{30} e LC_{80} apresentaram eficácia do extrato de sementes de *C. colocynthis* à base de metanol contra larvas de 1º instar de *E. vittella*. Os períodos larval e pupal aumentados foram revelados nas amostras tratadas durante a comparação com as amostras não tratadas. A taxa intrínseca de aumento e a taxa reprodutiva líquida nas concentrações de larvas expostas LC_{30} e LC_{80} permaneceram menores do que o tratamento controle. A fecundidade, a atividade da esterase e o conteúdo de proteína diminuíram nas amostras tratadas com LC_{30} e LC_{80} em comparação com o controle. As presentes descobertas sugerem que os extratos de *C. colocynthis* à base de inseticidas botânicos são benéficos, sustentáveis para o ecossistema e podem ser integrados com programas de manejo de insetos do ponto de vista da segurança ambiental.

Palavras-chave: meio ambiente sustentável, extratos vegetais, segurança do ecossistema, atividade da esterase, parâmetros da tabela de vida.

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1. Introduction

Spotted bollworm, *Earias vittella* Fabricius, 1794 (Noctuidae: Lepidoptera) is a global, polyphagous and most damaging pest of many economically important crops with wide host range which includes cotton, okra, hollyhock, China rose, shoe flower, sonchal and various other malvaceous plants (Syed et al., 2011). Spotted bollworm caused direct damage to fruiting bodies of cotton, *Gossypium hirsutum* and okra, *Abelmoschus esculentus* (Ahmad and Arif, 2009; Rahman et al., 2013). The larvae not only attack bolls and fruits though also damage the shoots, buds and flowers (Aziz et al., 2012). This notorious pest remained active throughout the year and at least 6 to 8 generations are observed each year. This pest causes up to 40% and 41.6% losses in seed cotton and okra, respectively whereas loss in cotton yield may reach up to 50% during severe infestation and larvae alone causes a damage between 50 to 70% including deformed fruits and low quality (Hassan and Ansari, 2010; Tanni et al., 2019).

Synthetic pesticides are the primary tools to manage insect pests of agriculture and veterinary. This sole dependence on chemicals, particularly in vegetables is not desirable due to health hazards. Furthermore, the injudicious exercise of chemicals has twisted numerous problems that jeopardize their efficiency further causing environmental pollution and intimidation to all biological and beneficial fauna (Hashmi, 1994). Recent years the indiscriminate use of insecticides leads to the development of resistance a big challenge for chemical control in insects (Ahmad et al., 2019; Bisset et al., 1997; Gulzar and Wright, 2015; Liu and Yue, 2000). It has been reported that many insect species have developed resistance against various insecticides (Whalon, 2008). The increasing concern on the development of resistance and restricted usage of pesticides has encouraged the application of bio-rational and botanical insecticides (Akhtar et al., 2012; Tome et al., 2013).

Botanicals or bio-pesticides can be an effective and advantageous replacement to commercial pesticides leading towards integrated pest approaches due to target-specific, eco-friendly, low number of applications and higher acceptable properties. Phytochemicals extracted from the plant parts by using different solutions have acted as toxicants, repellents and growth regulators (Ahmad et al., 2021; Amer and Mehlhorn, 2006; Choudhury et al., 2021). Synthesized chemical compounds produced by plants have the potentials to way forward to integrate insect pest control methods (Tabashnik, 1994). Plants have the aptitude to produce various bioactive substances and tributary metabolites such as alkaloids, glycosides, carbohydrates, terpenoids, tannins, phenols, flavonoids and sitosterols (Georges et al., 2008; Mann, 1978). These chemical components of plants are necessary for the actual value of mythological cures of entomological fauna. These components are extensively utilized in agriculture, veterinary scientific researches and numerous diversified arenas (Vasu et al., 2009). This plant-based stuff derived from stems, leaves, twigs, flowers, roots and fruiting portions has developed the part pesticides since ancient times (Cragg and Newman, 2001).

Citrullus colocynthis is generally recognized as the colocynth, bitter apple, desert gourd and viny plant (Cucurbitaceae) intrinsic to the Asia and Mediterranean arena and well established in the temperate areas of Pakistan. Many active chemical compounds (cucurbitacin A, B, C, D, E, I, J, K, L, glucosides I, L and flavonoids) from *C. colocynthis* have been described in previous studies (Delazar et al., 2006; Sturm and Schneider, 2009). Isolated compounds exhibited diverse actions against insect pests especially the compound cucurbitacin B is responsible for reduced oviposition and anti-feeding activity (Seenivasan et al., 2004;Tallamy et al., 1997) and these are also exploited as an abortifacient (Madari and Jacobs, 2004). These chemical compounds can manage and control various insect pests (Ahmad, 2007). It's almost two millennia that plant-mediated pesticides are being used in agriculture (Thacker, 2002).

Many investigators explored the effects of different plant extracts on the life span, fertility, fecundity and physiology of insects (Baskar et al., 2015; Pavunraj et al., 2016). *C. colocynthis* leaves extract with ethyl acetate, benzene, petroleum ether and methanol was commenced very effectively in populations of mosquito, *C. quinquefasiatus* (Mullai and Jebanesan, 2007).

The present study investigated the lethal and sub-lethal effects of the methanol-based extracts of *C. colocynthis* various biological and physiological factors of *E. vittella.*

2. Materials and Methods

2.1. Insect culture and sampling

Laboratory culture of spotted bollworm (*E. vittella*) was established from the larvae collected from okra and cotton fields in the surrounding three districts (Rawalpindi, Taxila and Attock) of Punjab province, Pakistan for the continuous three years (2018-2020). The infested pods from the fields were transferred into transparent plastic jars (20 cm length and 10 cm wide) and were retained within standardized conditions (27 ± 2 ℃, 60 ± 5% R.H., 16 hours light, 08 hours dark). Fresh Okra *(Abelmoschus esculentus)* fruits were utilized for sustaining the insect culture (Al-Mehmmady, 2000). Fresh okra fruits were thoroughly washed in clean water and desiccated before feeding to the neonates, and latterly these were cut into 0.5-1.0 cm pieces; 4-5 larvae were released per piece and placed in the plastic container. The food was replaced daily till pupation. The larvae were carefully removed from the okra pods and the excreta were cleaned. The pupae were shifted to another plastic jar (10 cm length and 5 cm width) until adult emergence. The emerged adults were shifted to adult cages (length x height x width: 38 x 34 x 30 cm) and nourished with 10% sugar solution. Nappy strips were provided in cages for oviposition and these strips were replaced regularly after observation of eggs.

2.2. Collection of seed and solvent extraction

C. colosynthis plant seeds were collected from various crop fields throughout the temperate regions of the Punjab province, Pakistan. Impurities from the collected seeds were removed manually and kept for drying under room temperature (Memon et al., 2003). The desiccated seeds were crushed in the shape of elusive powder with an automatic grinder. Fifty grams of the ground seeds were drenched in 100 ml of methanol, ethyl acetate, hexane and ethanol discretely for 24 h for each solvent extract separately. The mixture was stirred for 1 h and kept below 4°C in the refrigerator for 2 days. These refrigerated mixtures were stirred again for 1 h. These mixtures were filtered twice through filter paper and finally dissolved in 10 ml of their particular solvent to be conserved as a stock solution.

2.3. Phytochemical analysis

Different solvent extracts (methanol, ethanol and hexane) were utilized to carry out the preliminary phytochemical analysis of *C. colocynthis* with already standardized methodologies to recognize the constituents (Harbone, 1973).

2.4. Bioassays

Through the diet emersion method, bioassays were performed and toxicity was measured for 1st larval instar larvae of spotted bollworm. Different concentrations of *C. colosynthis* were prepared using different solvents (methanol, ethanol, hexane). Fresh okra fruits were dipped separately in each concentration for 10 seconds and then desiccated for 10 minutes at room temperature. Five okra fruits were used in one replication. Two 1st instar larvae were released on each okra pod in all replications treated with different concentrations. Four replications were performed for each treatment. The control treatment was served with purified distilled water. The entire treated larvae were kept under standardized lab conditions (25 ± 2 °C; RH 65 ± 5%, 16 hours light, 08 hours dark) and datasets were recorded regarding larval mortality at 72 hours.

2.5. Sub-lethal effects of plant seed extracts on biological on biological parameters of Earias vittella

Age-stage, two-sex life table theory was considered to estimate the stuff of sub-lethal concentrations on the biological factors of *E. vittella*. For life table study, 210 eggs were used, which were collected after 24 hours of deposition by females of the laboratory population. Three treatments (control, LC_{30} and LC_{80}) of methanolic extract were prepared for this experiment. Seventy eggs were treated with each treatment. Each egg in an individual Petri dish was reflected as a single replicate (Zhang et al., 2015; Chi and Yang, 2003). All petri dishes were kept under standardised conditions. The egg hatching data were recorded daily and the neonates from control, LC_{30}, LC_{50} and LC_{80} were shifted on the okra pods. The larval development was observed daily and fresh okra pods were provided after each instar. Pupae were removed and placed in new petri dishes until adult emergence. Then, the newly emerged adults were paired (one male and one female) and transferred to an individual plastic container for oviposition. The adults were patterned for oviposition and transported to fresh containers for egg-laying daily. The fecundity (%) and survival rate of the adults were assessed until the death of the adults.

2.6. Assays for esterase activity and protein content

The general esterase activity was determined through the method proposed by Van-Asperen (1962). In this experiment, 10 mM of α-naphthyl-acetate (αNA) and β-naphthyl-acetate (βNA) were treated as substrates. The frozen supernatant from the above treatments was transported to a fresh micro tube and phosphate buffer was added for making dilution. 1mM Fast Blue RR salt was also supplemented to this solution tube. The calculation regarding the absorbance was retained at 630 nm. Bovine Serum Albumin was utilized as a standard for the determination of the total protein contents (Bradford, 1976).

2.7. Data analysis

The LC values were calculated based on mortality data using R Statistical Software version 2.9.0 (R Development Core Team, 2018) The datasets regarding different developmental stage periods, survival rate and fecundity were scrutinized with the help of model age-stage, two-sex life table, theory and software (Chi, 1988, 2021). The means of the biological parameters were compared by using 100,000 bootstrap techniques to achieve stable standard error estimates (Huang and Chi, 2012). The curves for age-specific survival rate, life expectancy, fecundity and reproductive values were generated by using Sigma Plot 14.0. The net reproductive rate was calculated using the following Formula 1:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \tag{1}$$

The intrinsic rate of increase (r) was calculated by using the iterative bisection method through Formula 2:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \tag{2}$$

With age indexed from zero (Goodman, 1982) the mean generation time (T) was calculated by the Formula 3:

$$T = \frac{\ln R_0}{r} \tag{3}$$

The Gross reproductive rate (GRR) was calculated by the Formula 4 as follow:

$$GRR = \sum_{x=0}^{\infty} m_x \tag{4}$$

The age-specific survival rate (l_x) and age-specific fecundity (m_x) were given as (Formula 5 and 6):

$$l_x = \sum_{j=1}^{k} s_{xj} \tag{5}$$

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}} \tag{6}$$

3. Results

3.1. Toxicity Bioassays

The *Citrullus colosynthis* plant seed extract prepared in diverse solvents (methanol, ethanol and hexane) was treated against immatures (1st instar) of *E. vittella*. The LC_{30}, LC_{50} and LC_{80} values of methanol-based plant extract of *C. colosynthis* were calculated as (1.85, 4.60, 11.45), ethanol-based extract (3.29, 5.40, 8.85), hexane--based extract (3.57, 7.01, 13.78), profenofos (0.31, 1.74, 9.42) as positive control and water (3.06, 7.80, 19.89), respectively after 72 h of treatment (Table 1).

3.2. Lethal and sub-lethal effect of methanolic extract of C. colosynthis fruit on life tables of E. vittella

The larval development period was recorded from 1st instar to pupation stage of *E. vittella* treated with LC_{80} and LC_{30} as lethal and sub-lethal concentrations. The data of treated insects showed significant variation when compared with the untreated (Table 2, $p<0.001$, *df*=2 F=83.26). The

Table 1. Efficiency of *C. colosynthis* seed extracts in different solvents for 1st larval instar of *E. vitella.*

Solvent	N*	LC_{30} (95% CL)	LC_{50} (95% CL)	LC_{80} (95% CL)	Slope ± SE	χ^2(*df*)
Methanol	200	1.85	4.60	11.45	1.205 ± 0.0499	1.08 (6)
		(0.97 - 3.51)	(3.37 - 6.28)	(8.81-14.87)		
Ethanol	200	3.29	5.40	8.85	2.2195 ± 0.5137	0.98 (6)
		(2.50 - 4.33)	(4.44 - 6.56)	(6.43 -12.17)		
Hexane	200	3.57	7.01	13.78	1.625 ± 0.476	1.02 (5)
		(2.56 - 4.96)	(5.14 - 9.56)	(7.41-25.62)		
Water (NC)	200	3.06	7.80	19.89	1.173 ± 0.424	0.87 (6)
		(1.90 - 4.92)	(4.88 -12.47)	(6.98 -56.68)		
Profenofos (PC)	200	0.31	1.74	9.42	0.6497 ± 0.136	1.10 (5)
		(0.11- 0.95)	(0.88 -3.43)	(4.05-21.95)		

NC (negative control), PC (positive control), LC (lethal concentration), CL (confidence level), SE (standard error), *df (*degree of freedom), (Chi square test) χ^2. *Number of insects exposed.

Table 2. Life table parameters of *E. vittella* treated with sub-lethal concentrations of methanolic extract of *C. colosynthis.*

Treatments	Control		LC_{30}		LC_{80}		P (*df*)	F
	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE		
Egg (days)	70	3.66 ± 0.1^{b}	70	4.07 ± 0.094^{a}	70	4.26 ± 0.125^{a}	<0.001 (2)	107.8
1st instar (days)	64	2.28 ± 0.08^{c}	60	2.33 ± 0.088^{b}	58	2.52 ± 0.094^{a}	<0.001 (2)	67.44
2nd instar(days)	54	2.19 ± 0.076^{b}	50	2.20 ± 0.082^{b}	56	2.54 ± 0.096^{a}	<0.001 (2)	83.26
3rd instar (days)	48	2.17 ± 0.078^{b}	44	2.36 ± 0.105^{a}	46	2.48 ± 0.106^{a}	<0.001 (2)	41.87
4th instar (days)	46	2.13 ± 0.072^{b}	42	2.19 ± 0.088^{b}	40	2.53 ± 0.125^{a}	<0.001 (2)	63.24
5th instar (days)	46	2.26 ± 0.094^{b}	40	2.15 ± 0.082^{b}	38	2.47 ± 0.125^{a}	<0.001 (2)	86.91
Larva (days)	46	11.03 ± 0.028^{c}	40	11.23 ± 0.041^{b}	38	12.54 ± 0.013^{a}	<0.001 (2)	233.4
Pupa (days)	41	8.74 ± 0.201^{b}	34	11.35 ± 0.15^{a}	31	11.47 ± 0.151^{a}	<0.001 (2)	94.19
Total immature development	41	30.8 ± 1.76 ^{a}	34	33.81 ± 1.48 ^{a}	31	36.55 ± 2.04 ^{a}	<0.001 (2)	118.2
Male longevity (days)	18	8.52 ± 2.066^{a}	19	7.72 ± 1.56^{a}	16	7.12 ± 0.331^{a}	<0.001 (2)	41.23
Female longevity (days)	23	12.7 ± 2.13^{a}	15	9.04 ± 1.21^{b}	14	7.62 ± 0.242^{c}	<0.001 (2)	174.7
APOP	23	1.2 ± 0.133^{a}	15	1.0 ± 0.00^{a}	14	1.00 ± 0.00^{a}	<0.001 (2)	79.94
TPOP	23	24.5 ± 0.619^{b}	15	28.3 ± 0.26^{a}	14	29.22 ± 0.464^{a}	<0.001 (2)	79.94
Fecundity (eggs/f)	23	330.9 ± 26.41^{a}	15	268.5 ± 19.67^{b}	14	238.11 ± 14.24^{c}	<0.001 (2)	62.93

Adult pre oviposition period (APOP), total pre oviposition period (TPOP), LC (lethal concentration), n (number of samples), SE (standard error), *df (*degree of freedom), means sharing similar letters in a row are not different statistical at 5% probability.

maximum hatching duration (4.26 days) was recorded in LC_{80} treatment, which was non-significant to LC_{30} treatment (4.07 days) but significant (Table 2, p<0.001, *df*=2, F=107.8) to untreated ones (3.66 days). A significant variation was observed in the first to fifth larval instars. All larval instars show significant dissimilarities regarding duration of larval life (larva days) among lethal and sub-lethal treatments when compared with the untreated (Table 2, p<0.001, *df*=2, F=233.4). Maximum larval duration (12.54 days) was recorded in LC_{80} treatment followed by LC_{30} (11.23 days) while minimum larval days were taken by the untreated larvae (11.03 days). Mean pupal duration was found to be significantly longer in larvae treated with LC_{80} and LC_{30} concentrations compared to the control (Table 2, p<0.001, *df*=2, F=94.19). The solicitation of lethal and sub-lethal concentrations significantly enhanced the total developmental period of *E. vittella* from 1st instar to adult as compared to the untreated (Table 2, p<0.001, *df*=2, F=118.2). Female longevity was affected more than male longevity and the maximum female longevity was recorded in the control treatment (12.7 days) which was significant to sub-lethal (LC_{30}) and lethal (LC_{80}) concentrations (Table 2, p<0.001, *df*=2, F=174.7). While male longevity was not altered significantly between the applied treatments (Table 2, p<0.001, *df*=2, F=41.23). Non-significant trends were observed between all the treatments in adult pre oviposition period (APOP) and total pre oviposition period (TPOP) of *E. vittella* (Table 2, p<0.001, *df*=2, F=79.94).

The average pupal weight of the insects exposed to lethal and sub-lethal concentrations was lower than the control treatment (Table 3, p<0.001, *df*=2, F=204.1). The percent pupation after LC_{80} treatment (52.48) was less than LC_{30} (58.52) and control (91.75) treatments (Table 3, p<0.001, *df*=2, F=11.93). The lethal and sub-lethal concentrations were recorded to reduce the percentage of adult emergence significantly with comparison to the control treatment (Table 3, p<0.001, *df*=2, F=21.49).

The application of a methanol-based extract of *C. colosynthis* at lethal, sub-lethal and control concentrations (67, 41 and 89) resulted in the reduction of the mating pair's success (Table 3, p<0.001, *df*=2, F=20.05). A significant decrease in the fecundity egg/female was perceived laid by each female in the lethal and sub-lethal treated population with comparison to control (Table 2, p<0.001, *df*=2, F=62.93). The hatchability percentage of eggs (viability) was also affected and reduced significantly in the lethal and sub-lethal treated populations when compared to the control treatment (Table 3, p<0.001, *df*=2, F=266.81).

C. colosynthis plant extracts significantly altered the population parameters of the *E. vittella* (Table 4). To estimate the population parameters, the bootstrap method with 100,000 replicate was applied. The increased intrinsic rate was decreased by treating with both the concentrations of *C. colosynthis* plant extract (0.128 d^{-1} and 0.140 d^{-1} for LC_{80} and LC_{30} respectively) as compared with the untreated larvae (0.166 d^{-1}). Similarly, the trend was exhibited in the finite rate of increase (λ), as the highest value for λ was observed in control larvae (1.181 d^{-1}), which gradually decreases with an increase in concentration from LC_{30} to LC_{80} as 1.150 d^{-1} and 1.137 d^{-1} respectively. A significant decrease was also observed in the net reproductive rate (Ro) after treatment from being highest in control (94.542) to 76.714 and 61.228 offsprings/individual for LC_{30} and LC_{80} treated larvae, respectively. The highest Gross reproductive rate (GRR) was recorded for control larvae (183.42), while the lowest for LC_{80} treated larvae (136.77) progeny/individual which was both statistically different to each other. The mean generation time was prolonged in the treated larvae in comparison with the larvae of control treatment. Minimum mean generation time was taken by the control larvae (27.255 d^{-1}), followed by LC_{30} treated larvae (30.967 d^{-1}). The maximum days were recorded on LC_{80} treated larvae (31.917 d^{-1}). The S_{xj} represents the age-stage survival rate, it shows the chances of afresh laid eggs to survive till (age x and stage j) as shown in (Figure 1). Significant variations were observed in the growth rate of the various entities in the population (Figure 1). The lethal and sub-lethal treatments show overlapping curves of the developmental stages as compared to control. Each developmental stage showed a different pattern of the peak curves for lethal and sub-lethal of *C. colosynthis* plant extract and control. The curve line for the male adults ends earlier (Figure 1) as compared to female adult lines. Survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) were calculated for lethal and sub-lethal concentrations for *E. vittella* (Figure 2). A significant decline of l_x curves was observed in the lethal and sub-lethal treated population much earlier than that in control. Higher peaks for f_x curves were noted in control, while much lower f_x curves were noted in LC_{80} and LC_{30} treated populations. The e_{xj} (life

Table 3. Mean biological parameters of *E. vittella* treated with lethal and sub-lethal concentrations of methanolic extract of *C. colosynthis.*

Treatment	No. of Pairs	Mating of pair success%	Percent Pupation	Percent Exclusion	Pupal weight	Adult emergence	%Viability of eggs ± SE
Control	9	89[a]	91.75 ± 2.70[a]	91.75 ± 2.70[a]	63 ± 0.72[a]	41 ± 2.84[a]	85 ± 1.98[a]
LC_{30}	9	67[b]	58.52 ± 5.22[b]	49.75 ± 4.57[b]	41.23 ± 0.46[b]	34 ± 1.71[b]	70.4 ± 3.27[b]
LC_{80}	9	41[b]	52.48 ± 3.14[c]	60.5 ± 3.17[c]	52.48 ± 0.56[c]	30 ± 1.09[c]	75.89 ± 2.59[b]
P (*df*)		<0.001 (2)	<0.001 (2)	<0.001 (2)	<0.001 (2)	<0.001 (2)	<0.001 (2)
F		20.05	11.93	167.8	204.1	21.49	266.81

SE (standard error), *df (*degree of freedom), Similar letters with means in columns indicate a non-significant difference at probability level <0.05.

Table 4. Effect of sub-lethal concentration of methanolic extract of *C. colosynthis* on the biological parameters of *E. vittella*.

Population parameters	Control		LC_{30}		LC_{80}	
	Mean	SE	Mean	SE	Mean	SE
Intrinsic rate of increase (*r*) d^{-1}	0.166[a]	0.011	0.140[b]	0.009	0.128[c]	0.010
Finite rate of increase (λ) d^{-1}	1.181[a]	0.013	1.150[b]	0.010	1.137[b]	0.011
Net reproductive rate (R_o) d^{-1}	94.542[a]	25.23	76.714[ab]	20.679	61.228[b]	17.610
Mean generation time (*T*) d^{-1}	27.255[c]	0.573	30.967[b]	0.272	31.917[a]	0.301
Gross reproductive rate (*GRR*) d^{-1}	183.42[a]	8.055	146.45[ab]	32.83	136.77[b]	30.553

LC (lethal concentration), SE (standard error), means sharing similar letters in a row are not different statistical at 5% probability.

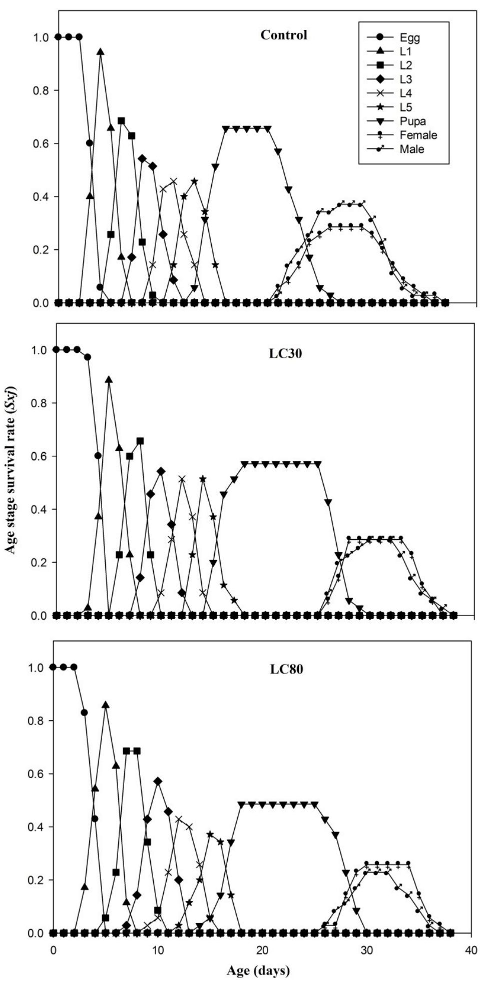

Figure 1. Age-stage survival rate (S_{xj}) of *E. vittella* treated with LC_{30} and LC_{80} of *C. colosynthis* extract.

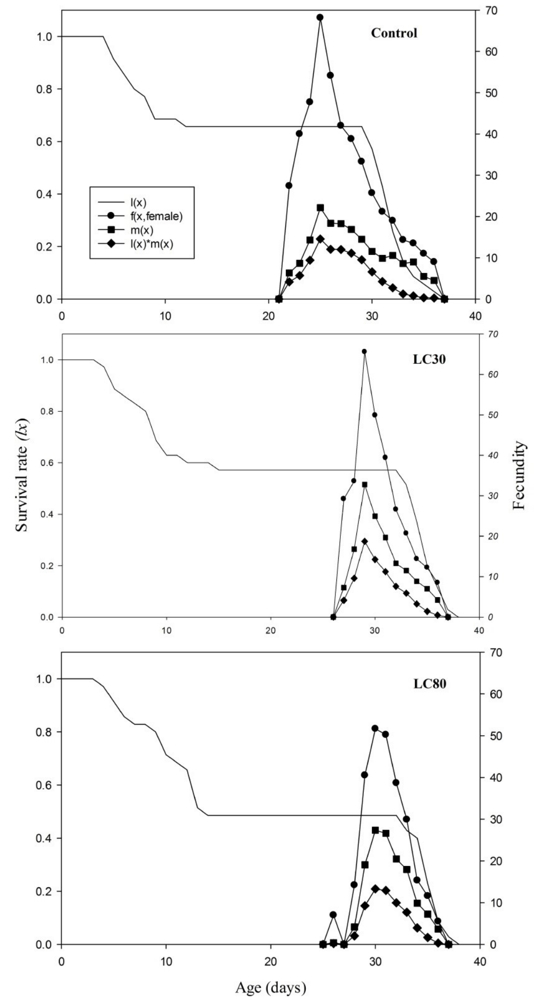

Figure 2. Survival rate (l_x), Age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *E. vittella* treated with LC_{30} and LC_{80} of *C. colosynthis* extract.

expectancy) curves varied significantly among the treated (LC_{80} and LC_{30}) and untreated for *E. vittella* (Figure 3). The minimum life expectancy for eggs was observed on the lethal, followed by sub-lethal, while the maximum value of life expectancy for eggs was observed in the control. Reproductive rate (vxj) is defined as the measure of dedication to newly coming offspring in the future from age x to stage j (Figure 4). The contribution of males in the population to the next generation was not well defined; therefore the curve for males was not included. A decline

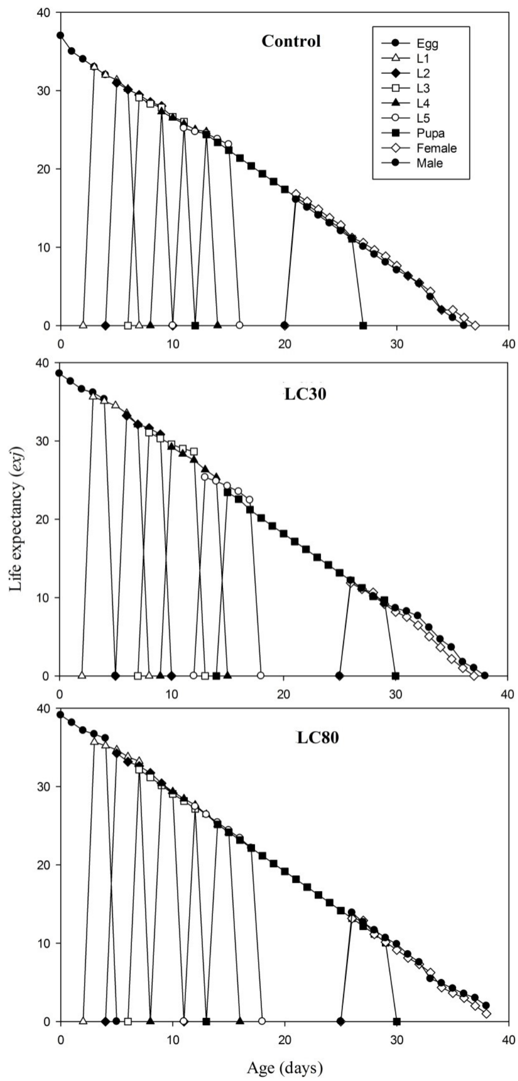

Figure 3. Life expectancy (e_{xj}) of *E. vittella* treated with LC_{30} and LC_{80} of *C. colosynthis* extract.

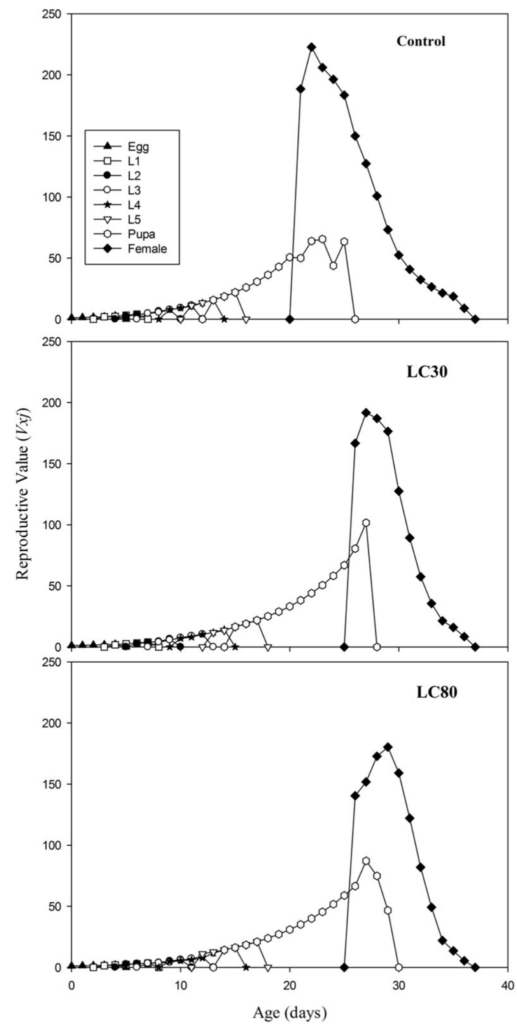

Figure 4. Age-stage specific reproductive value (V_{xj}) of *E. vittella* treated with LC_{30} and LC_{80} of *C. colosynthis* extract.

Table 5. Esterase activities and total protein content in *E. vitella* in methanolic extract of *C. colosynthis.*

Treatments	Esterase Activity (μgmol / min/ mg protein)	% Reduction	Total Protein (mg/ml)	%Reduction
LC_{30}	0.132 ± 0 .085	45.46 ± 0.085	203.94 ± 2.58	26.10 ± 2.58
LC_{80}	0.094 ± 0.001	61.16 ± 0.001	145.794 ± 1.06	47.17 ± 1.06
Control	0.242 ± 0.002	- -	275.96 ± 1.47	- -
P (*df*)	<0.001 (2)	-	<0.001 (2)	-
F	1704.2	-	349.5	-

LC (lethal concentration), *df (*degree of freedom).

was observed in the reproductive values when larvae were treated with LC_{80} and LC_{30} as compared to the untreated larvae. Maximum vxj was recorded on the untreated group, while the minimum was observed in the LC_{80} treated group.

3.3. Total protein content and esterase activity of E. vittella treated with plant extract

Total protein content in *E. vittella* treated with lethal and sub-lethal deliberations of *C. colosynthis* plant extract were shortened to a significant level as compared to the untreated treatment (Table 5, p<0.001, df=2, F=349.5). The average esterase enzyme activity in *E. vittella* treated with lethal and sub-lethal concentrations was significantly reduced when compared with the untreated control populations (Table 5, p<0.001, df=2, F=1704.2).

4. Discussion

In the present study, the presentation of lethal and sub-lethal deliberations of *C. colosynthis* seed mines significantly influences the developmental time, pupal weight, pupal percentage, adult emergence and adult longevity. The methanolic extract of *C. colosynthis* prolonged larval development, pupal developmental time as compared with the control. Ramzi et al. (2015) also described related results, when the caterpillars of *Apomyelois ceratoniae* were pickled with *C. crenata* as compared with the populace of control treatment, this difference might be the metamorphosis interruption grounds.

The auspicious results of *C. colosynthis* to control *E. vittella* might be due to the presence of compound cucurbitacin B and responsible for the reduced hatchability antifeedant activity (Tallamy et al., 1997). The present study reported that *C. colocynthis* seed extract significantly affects the mating, fecundity and egg viability in *E. vittella* treated with lethal and sub-lethal concentrations. Such kind of results was also described by various researchers (D'Incao et al., 2012; Khani et al., 2013; Khosravi et al., 2011). Lethal and sub lethal effects along with reduced fecundity and fertility rates were reported in *Spodoptra frugiperda* when treated with saponins extract, obtained from *Passiflora alata* (D'Incao et al., 2012). The extract of *Piper nigrum* and *Jatropha curcas* reduced the egg viability and adult emergence of *C. cephalonica* with control comparison (Khani et al., 2013).

The intrinsic rate of natural increase (rm) provides an estimate of the growth probability of insect populations (Rabinovich, 1972) which can offer great perception into the population growth of species besides other life-history factors. Whereas it is also conceivable to estimate the net rate of reproduction (R_o) dependency of natural increase in the intrinsic rate principally based on productiveness, % hatching, growth and adult emergence (Khan et al., 2013; Saeed et al., 2010). Aiming the purpose, the above mentioned divergent life-history abilities might simulate the population increase rate of *E. vittella*. The intrinsic rate of natural increase (r_m) was significantly declined in lethal and sub-lethal concentrations as compared with control treatment. These findings are also described by Hafeez et al. (2019) in a way that the intrinsic rate of increase was significantly changed due to lethal and sub-lethal concentrations of lufenuron whereas present study tested plant extracts also showed the comparable results. Correspondingly, the average generation time was significantly protracted in the treated population equal to the mean generation time of the untreated population. The escalation was observed in the mean generation time of *S. litura* when treated with different doses of imidacloprid (Abbas et al., 2012). Our results exhibit that parameters of the life table of *E. vittella* were adversely transformed with the lethal and sub-lethal concentrations of *C. colocynthis* plant extracts. A significant change was found in the S_{xj}, which decreased in the treated population compared to the untreated population. f_{xj} and m_x were also found to be decreased in the lethal treated population as compared to the control population. A measure of the influence of new individuals in population growth (e_{xj}) declined sharply in second larval instars in the lethal and sub-lethal treated population. The methanolic extract of *C. colosynthis* significantly reduced the esterase activities in larvae of *E. vittella*. The reduction in the esterase activity which is a vital detoxifying mechanism may indications to a substantial control of various insect pests (War et al., 2014). Equivalent results about the retarded esterase activity due to plant extract in insects were perceived (Khosravi et al., 2011). Furthermore, by applying the *C. colocynthis* extracts on 1st larval instar of *E. vittella* reduction in total protein contents was observed. Similar reduction in the activity of α-amylase and deterioration of protease was observed when compared with the control in *Glyphodes pyloalis* (Khosravi et al., 2011). The decrease in protein content might be due to the interruption intention in ATP synthesis. These insecticidal activities might be due to individual efficacy or the synergistic action of biological compounds present in these plants.

5. Conclusion

The methanol extract of *C. colosynthsis* displayed toxic properties on the development and survival of *E. vittella*. The present results suggested that plant extract-based control measures may be integrated into the pest management programs with special emphasis on the environmental tactics for ecosystem safety.

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