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The Potency Of Ricinine Biopesticide From *Ricinus communis* Leaves As An Alternative Host For Mass Rearing Process Of *Tetranychus urticae* And Two Predatory Phytoseiid Mites

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Abstract

The aim of this investigation was to develop an easier and cheaper alternative method for rearing the predatory phytoseiid mites, *Phytoseiulus persimilis* (Athias-Henriot) and *Neoseiulus californicus* (McGregor), as biological control agents of *Tetranychus urticae* Koch under plastic tunnel conditions in the Egyptian new reclaimed area. The castor bean (*Ricinus communis* L.) shrub was the species tested because it appears to be a less expensive to grow and more tolerant alternative host plant to the common bean, *Phaseolus vulgaris* L. However, such proposed host plants have got an efficient alkaloid marked by its insecticidal effect. The biology of both predatory mites and their prey were studied under laboratory conditions at 27 °C ± 2 and 70% ±1humidity. The developmental times and reproduction rates of *T. urticae* and *P. persimilis* were significantly affected by *R. communis* cultivars, while such differences was not detected for *N. californicus*. The total alkaloid ricinine amounted in the leaf crude extract powder 88 and 66 mg /g in red and green leaves of castor bean respectively. Ricinine percentage yield in both cultivars reached 0.59% in green and 0.49% in red leaves respectively.

Keywords: *Ricinus cummunis; land races; phytoseiid mites; life table; Tetranychus urticae; ricinine alkaloid*

Introduction

Ricinus communis L (RC) belongs to Euphorbiaceae family and is also called castor bean. India is the second largest producer of castor seed in the world. The main secondary metabolites of RC are alkaloids, squalene, sitosterol, anthocyanin, tocopherols and fatty acids such as ricinoleic, linoleic, and stearic acid. The Leaves of RC is used as medicine against intestinal worms, strangury, night blindness, etc. The leaves change from pale green to dark red based on the content of anthocyanin pigmentation and different concentrations of others metabolites [1].

Ricinus communis is known as wonder tree and is considered an important traditional medicine plant in

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Egypt. It is a perennial shrub that probably originates from Africa and was used in ancient Egypt and by the Romans and Greeks Scarpa and Guerci [2]. It has been used to control insect pests in several crops as it showed larvicidal and insecticidal activities against the adult of *Haemaphysalis bispinosa* Neumann and the hematophagous fly *Hippobosca maculata* Leach (Diptera: Hippoboscidae) [3, 4].

Biopesticides are alternatives to synthetic pesticides, since they are known to have the advantage of low toxicity to the ecosystem and segregate quickly in the environment [5]. Numerous plant extracts or oil, called biopesticides, are available without indications of the bioactive compounds or the target pests. Ricinine is known as a bio- insecticide [5]. Alkaloid is one of the major active compounds in castor bean residues than in leaves [6].

Phytoseiid predatory mites are highly effective biocontrol agents of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). They prey on mites, small insects, nematodes, and fungi [7]. The majority of literature on acarine biocontrol agents pertains to the phytoseiid. About 20 species of phytoseiid are currently mass-reared and sold by 50 companies worldwide [8].

Culturing of predatory mites could be carried out in bulk for release purposes commercially. Mass rearing facilities should ensure that there are no contaminants with other species or pathogens and study the optimal conditions for their mass culture. The start population of *P. persimilis* should be kept under cross-breeding with exotic strains to avoid inbreeding depressions [9]. The mass-rearing of predatory mites usually requires the mass-rearing of the prey pests and their host plants; growing healthy plants is an essential step [10]. Legumes (beans, groundnuts or soya beans) are very suitable as hosts for spider mites. They are usually grown at 20–30°C, 50–80% RH and 12–16 h light (>1000 lux), with the appropriate fertilizers. Cultivars of the same host plant may differ in their suitability for the prey [12]. An efficient and less expensive method is the production of the predatory mites directly on plants, where their prey is reared. The castor bean (*R. communis*) shrub was the species under test as an alternative host plant to the common bean, *Phaseolus vulgaris L* because it is cheaper to grow and more tolerant to abiotic stresses [12].

The two-spotted spider mite *T. urticae* Koch s a serious pest worldwide, causing serious damage to vegetables, flowers, and fruit crops and particularly in protected cultivation in arid zones [13, 14]. It has been recorded inhabiting 1200 host plants species of which 10% are economically important. It may transmit many plant viruses [15]. In Egypt, *T. urticae* is damaging many host plant species, including cotton, corn, soybeans, many orchard crops, and ornamentals particularly those grown under plastic tunnel conditions [13]. It can significantly cause crop reduction or total loss [16]. This spider mite has developed serious resistance to many chemical pesticides [16, 17].

The present study is concerned with the chemical analysis of total alkaloids of both castor bean cultivars, and the biology and life table parameters of *T. urticae*, *N. persimilis* and *N. californicus* were studied using red and green cultivars of *R. communis*. To our knowledge, no works have been published on using castor bean leaves of both red and green

cultivars for rearing spider mites in the laboratory except some lepidopterous larvae.
[18-22].

MATERIALS AND METHODS

1. Chemical part

a. Solvents and chemicals

All solvents used were HPLC grade. Methanol, Ammonia and dichloromethane were purchased from Merck (Germany) and Deuterated NMR solvents ($CDCL_3$, CD_3OD).

b. Determination of total alkaloids from *Ricinus communis* leaves.

One hundred gram from *Ricinus communis* red and green type leaves were extracted with methanol was evaporated till dryness and weighed according to Kam et al., [23] and El-Gengaihi et al., [24].

c. **Isolation and identification of ricinine alkaloid from green type.**

Two kg dried powdered *Ricinus communis* (green type) from leaves was extracted with methanol was evaporated till dryness and weighed 105 g of the dried methanol extract of leaves was dissolved in acidified water 1% HCl. The acidified mixture was transferred to a separating funnel and extracted with chloroform until color free. The chloroform layer was discarded. The remaining acidified aqueous extract was filtered, and then converted to alkali by adding ammonia, and the pH was adjusted from 9 to 11. The aqueous alkali is extracted with chloroform. The combined chloroformic layer was evaporated to dryness, weighed (1.2g), mixed with about 2 g silica gel, then put on the top of silica gel ammoniated column (12.5mm x 150cm) and eluted with Choroform: methanol with increasing polarity till 100% MeOH [23, 24] .

Fractions of 50ml were pooled from the column, then checked by TLC with eluting system of CH_2CL_2: MeOH (9.5: 0.5, v/v). Similar fractions were combined together; detection of alkaloids was done by Dragendorff reagent.

Analysis of ricinine by ^{1}H-NMR, ^{13}C-NMR and LC-MS-MS:

Ricinine was identified by using NMR spectra (Central Services Lab. NRC, Egypt), with the following conditions:

Nuclear Magnetic Resonance (NMR): The ^{1}H-NMR and ^{13}C-NMR, spectra were measured using 500 MHz and 125 MHz spectrometers, respectively with internal standard TMS. (Jeol-ECA-500, Japan) [25].

Ultra Performance Liquid Chromatography- High Resolution Mass Spectrometry (UPLC-HRMS): UPLC (Waters, USA) system coupled with high resolution Q Exactive mass spectrometer (Thermo Fischer Scientific, Germany) at Poznan, Poland. Chromatographic separation for this system was carried out using water acidified with 0.1% formic acid (solvent A) and acetonitrile (solvent B). The mobile phase flow of 0.41 mL/min was adjusted to the following gradient: (0-9 min) from 50% A to 50% B, (9-17 min) to 98% B and maintained in these conditions for 5 min. then system returned to the starting conditions and was re-equilibrated for 3 min. with the BEH shield C18 column (150×2.1 mm, 1.7 μm). Q Exactive 2.3 (for tune application) software from Thermo Fisher Scientific (MA, USA) was used to control mass spectrometer. TF Xcalibur 3.0 software was utilized for data handling [26].

2. Biological studies:

The biological investigation was conducted in the National Research Centre, Egypt. All the experiments were carried out in an growth chamber at 27±1°C and 60-80% R.H. and a photoperiod of 16 L:8 D.

***Ricinus communis* cultivars:**

Seedlings of *R. communis* red and green cultivars were transplanted in plastic pots filled with beet moss and placed in a greenhouse. No pesticides and fertilizers were used, and the cultivated plants were reared according to the normal agriculture practices.

Culture of *Tetranychus urticae* :

Individuals of *T. urticae* were obtained from leaves of *R. communis* from Benha, Qaliubia Governorate. Culture of *T. urticae* is maintained on whole plants of each cultivar for at least 4 generations before starting the experiments.

Experiments:

For each cultivar, leaf discs (1.5 cm) cut from fully expanded leaves placed underside up on water-saturated cotton pads in Petri-dish 12 cm as experimental arena. Water-saturated cotton strip 0.25 cm was placed around the edge of the leaf discs to prevent mites from escaping. The rearing dishes were kept in an incubator at 27±1°C, 60-80% RH.

Newly emerged mated females of *T. urticae*, obtained from the stock colony of each cultivar, and were transferred into fresh leaf discs of each cultivar separately, so that oviposition could occur. After eight hours, females were removed and only one egg was kept on each leaf disc, from which the development was monitored. The presence of an exuvium was used as the criterion of successful molting to the next developmental stage. On the same cultivar, the newly emerged female was paired with a male. When it was necessary, young males from culture of the same cultivar were used for multiple mating with females. The number of laid eggs was evaluated daily until the female death; eggs were removed daily to avoid double counting.

Predatory phytoseiid mites colony:

Phytoseiulus persimilis and *N. californicus* cultures were initially obtained from mite rearing units in the NRC laboratory stock culture. For each cultivar, culture of each predatore was maintained separately on fully expanded leaves, placed underside up on water-saturated cotton pads in Petri dishes, provide water supply to prevent predators escaping [2] and maintain leaf freshness. Leaves were infested with spider mites and 24 h. later phytoseiid mites of each species were transferred onto the leaves. Every two days, *T. urticae* infested castor bean leaves were supplied as food. This culture started one month before the beginning of experiments. The rearing unit was maintained at 27±1°C, 60-80% RH in the growth chamber.

Experiments:

The experimental arena consisted of red and green leaf discs. On a water-saturated cotton pad in a 12 cm diameter Petri dish, discs (1.5 cm in diameter) was placed upside down , the borders of the units were surrounded by wet cotton strips (0.25 cm width) as barriers. In such experimental unit designs, leaves are easily replaceable with fresh ones. Experimental arena was held at laboratory conditions like predator culture.

Newly emerged mated females of each predatory mite were obtained randomly and transferred individually into the experimental units and supplied with *T. urticae* nymphs, consumed prey individuals were replaced by alive ones daily. After eight hours, females were removed and only one egg was kept on each leaf disc. Observations were made every day and developmental duration of egg and developmental stages were recorded for both female and male. After adult emergence, females and males

were paired and monitored daily to record death and reproduction. The laid eggs were removed and transferred to new experimental arenas to determine sex ratio of the offspring in each cultivar.

Statistical analysis:

Differences in the duration of different life stages and fecundity of the spider mite and the predatory mite were analyzed using t-test (SPSS:V.16).

Age-Stage, two-sex life table

Developmental times, survival, longevity and fecundity of all replicates were analyzed. Following Chi and Liu [28], age-stage specific survival rate (*sxj*) (where *x* is age and *j* is stage), age specific survival rate (*lx*), age-stage specific fecundity (*fxj*), age-specific fecundity (*mx*) and population parameters including : (*r*) the intrinsic rate of increase, (λ) the finite rate of increase, (R_0) the net Reproductive rate, (*GRR*) the Gross Reproductive Rate, (*T*) mean generation Time, were estimated based on age-stage and two-sex life table [28, 29] using TWOSEX-MS Chart software [30]. The age-specific survival rate (*lx*) and the age-specific fecundity (*mx*) for both female and male were calculated according to Chi and Liu [28].

Results:

Chemical Part:

Isolation of ricinine: chromoformic fraction of the leaves extract was found to be mainly composed of ricinine, which was crystallized out and further purified by recrystallization from CH_2CL_2-MeOH mixture (4:1, v/v). Ricinine was obtained as creamy white needles (0.89 g), with melting point 202°C and R_f value of 0.45 using CH_2CL_2-MeOH (9.5:0.5, v/v) as an eluting system on silica gel thin layer chromatography. The purity of ricinine was checked by TLC. The TLC chromatogram showed a single spot of ricinine that had a purple quenching at λ_{254} UV light and gave orange color when heated after spraying with Dragendorff reagent. Ricinine obtained in crystalized form as a white needle amounted to 0.59% of the crude chloroformic extract of the green type and was 0.49% of the red type.

Analysis of ricinine by ^{1}HNMR, ^{13}C-NMR and LC-MS/MS:

Ricinine ^{1}H NMR (500 MHz, $CDCl_3$): 7.55 (d, 1H, J =7.7 Hz, (H-6)), 6.08 (d, 1H, J= 7.7 Hz, (H-5)), 4.00 (s, 3H, CH_3O) and 3.53 (s, 3H, CH_3N) Figure (2B). ^{13}C NMR (125 MHz, $CDCl_3$): 37.6 (NCH_3), 57.2 (OCH_3), 77.35 (C-3), 93.65 (C-5), 113.76 (CN), 143.64 (C-6), 161.37 (C-2), 172.4 (C-4) Fig (2A). LC–MS/MS, high resolution ($[M+H]^+$ calculated: $C_8H_9N_2O_2$ as 165.0655 and measured as 165.0659, fragmentation ion; 183.0547, 110.0603, 84.0449, 68.0501) as seen in in figures Figure (1A) and Figure (1B). The identification of ricinine was performed using LC–MS/MS (Figures 1A and 1B), and 1H and 13C NMR (Figures 2A and 2B), by comparison with literature data [31].

Biological studies:

As shown in Table 1, *T. urticae* developmental periods were significantly influenced by cultivars. Life cycle on the red cultivar was longer than on the green cultivar reaching a value of 19.33 versus 14.92 days respectively ($P < 0.01$). The same trend was found in male life cycle of 16.58 and 12.25 days using the two cultivars, respectively. Female and male longevity were almost similar on both cultivars. Female life span varied significantly using the red and green cultivars and was 38.58 and 33.42 days, respectively ($P < 0.01$).

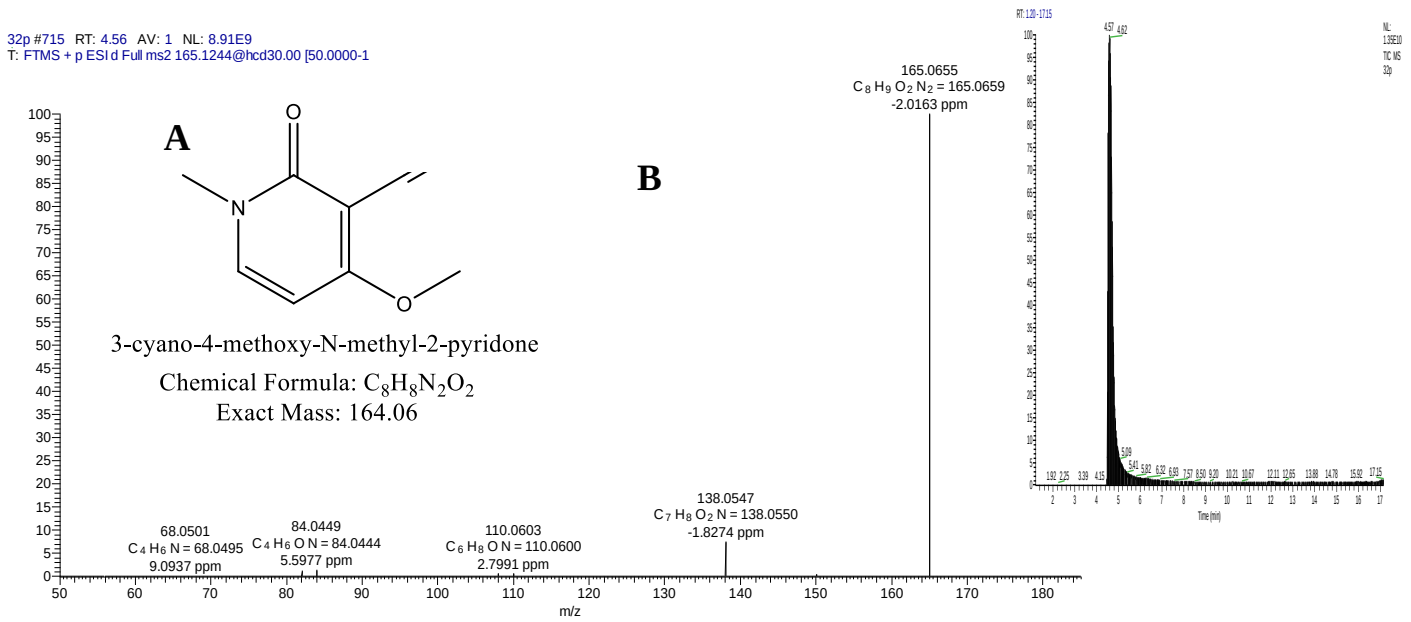

Figure 1: Ricinine alkaloid characterized by A) HR-MS/MS by $[M+H]^+$ mode and B) UPLC.

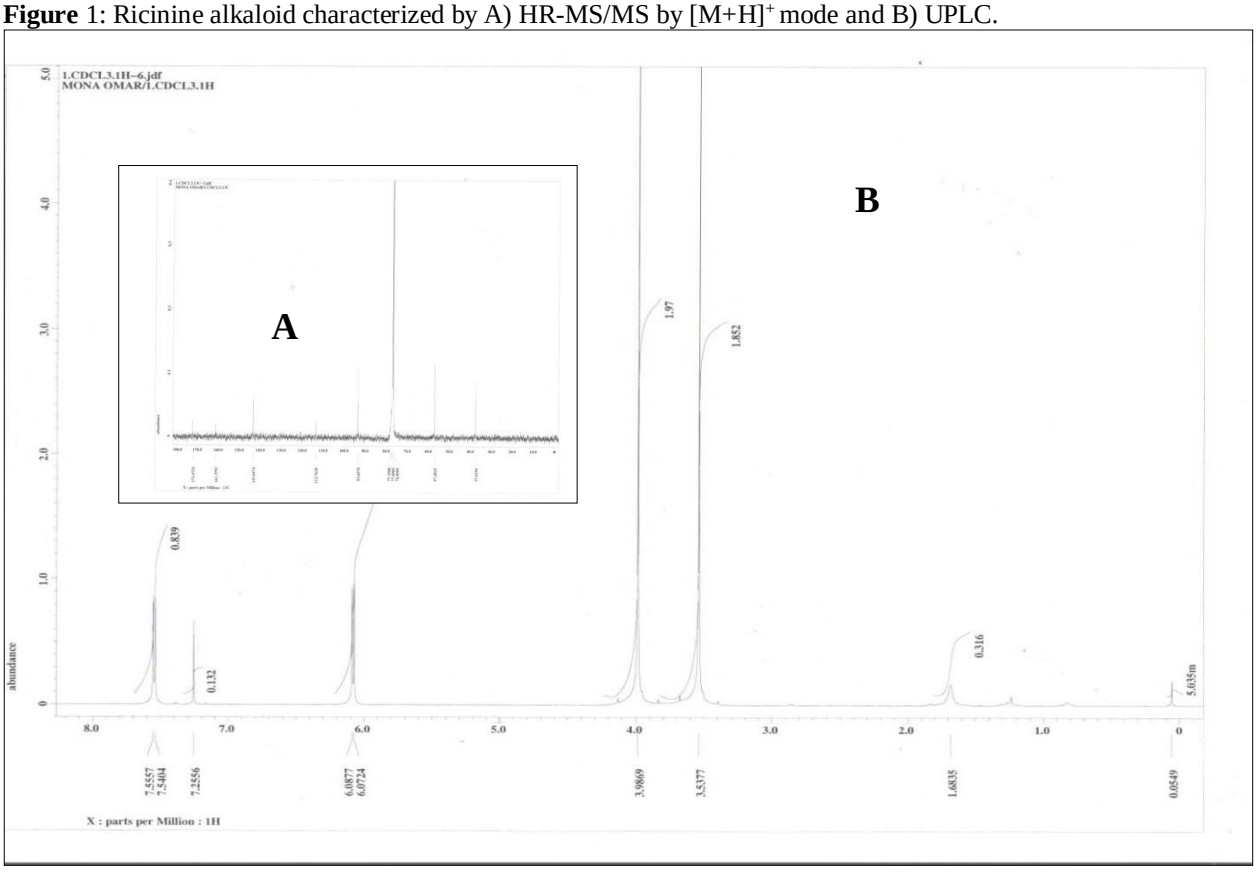

Figure 2: A) ^{13}C-NMR & B) ^{1}H-MNR of Ricinine compound.

Total and daily egg production was significantly reduced by rearing on the green cultivar, by laying a total of 55.08 comparing to 73.75 eggs/ female on red one ($P < 0.01$). Considering life table parameters of *T. urticae* on both red and green cultivars, Table 2 shows that there is no significant difference in r , λ and R_0 values (0.14, 1.15 and 36.88) (0.16, 1.17 and 27.58) respectively. Mean generation time (T) was significantly longer on the red cultivar 25.91 days and 21.09 days on the green cultivar ($P < 0.05$).

Table 1: Mean developmental time, and biological parameters (± SE) of *Tetranychus urticae* reared on *Ricinus communis* (Red and Green cultivars).

Developmental stages	Host plant		T value
	Red cultivar	Green cultivar	
Egg	5.58 ± 0.15a	4.33 ± 0.14b	6.078**
Larva	5.50 ± 0.15a	3.75 ± 0.18b	7.4667**
Protonymph	4.00 ± 0.00a	2.83 ± 0.21b	5.631**
Deutonymph	4.25 ± 0.13a	4.00 ± 0.00a	1.915 ns
Female life cycle	19.33 ± 0.14a	14.92 ± 0.19b	18.427**
Male life cycle	16.58 ± 0.38	12.25 ± 0.43	
Pre-oviposition period	2.00 ± 0.00a	2.00 ± 0.00a	0.000
Oviposition period	15.67 ± 0.40a	14.67 ± 0.53a	1.517 ns
Post-oviposition period	1.58 ± 0.15a	1.83 ± 0.21a	0.980 ns
Female longevity	19.25 ± 0.35a	18.50 ± 0.48a	1.254 ns
Male longevity	18.17 ± 0.51	18.08 ± 0.47	
Female lifespan	38.58 ± 0.40a	33.42 ± 0.54b	7.674**
Male lifespan	34.75 ± 0.54	30.33 ± 0.43	
Fecundity	73.75 ± 3.03a	55.08 ± 1.91b	5.212**
Egg/♀ / day	4.71 ± 0.15a	3.77 ± 0.10b	5.251**

The means in each row with the same letters are not significantly different (t-test, SPSS) ns= no significans, **p< 0.01 .

Table 2: Life table parameters (mean ± SE) of *Tetranychus urticae* reared on *Ricinus communis* (Red and Green cultivars).

Life table parameters	Host plant	
	Red cultivar	Green cultivar
Intrinsic rate of increase (r)	0.14±0.01 a	0.16±0.01 a
Finite rate of increase (λ)	1.15±0.01 a	1.17±0.01 a
Net reproductive rate (R_0)	36.88±6.81 a	27.58±5.57 a
Mean generation time (T)	25.91±0.22 a	21.09±0.40 b
Gross reproductive rate (GRR)	38.95±7.22 a	32.73±7.00 a

Data in each cultivar is related to the age-stage, two-sex life table. Mean values in a row followed by different letters are significantly different (Paired bootstrap test, $P \leq 0.05$).

As seen for age specific survival and fecundity of *T. urticae* reared on red and green cultivars (Figure 3). The survival percentage of female *T. urticae* using the green cultivar was very high until day 27 then, started gradually to decline. Whilst egg laying started on day 15, peaking on day 21. On the red cultivar, *T. urticae* survival ship was very high and mortality started on day 33, almost a long female longevity. The egg laying started on day 20, peaking on day 26 then declining gradually almost similar to the former increasing rate.

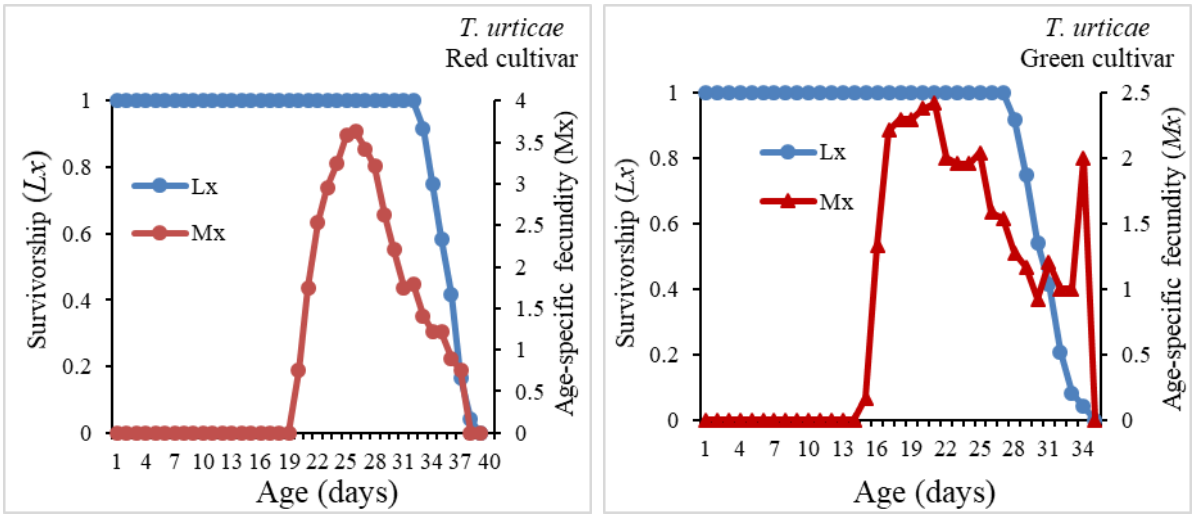

Figure 3: Figure 3. Age-specific fecundity (*Mx*) and survivorship (*Lx*) of *Tetranychus urticae* reared on *Ricinus communis* (Red and Green cultivars).

Predatory mites:

Female of *P. persimilis* reached the end of life cycle in 7.25 and 8.00 days on red and green cultivars, respectively ($P < 0.01$). Cultivars significantly affected the duration of oviposition period ($P = 0.05$), but no significant difference was observed for pre-oviposition and post-oviposition periods. Duration of female life span on the two cultivars was also very close 37.83 and 36.83 days, respectively. While female fecundity varied significantly of 53.42 , 48.52 eggs/female on red and green cultavers, respectively ($P < 0.01$) (Table 3).

No significant difference was recorded for durations of the developmental stages for *N californicus*, life cycle reached 8.33 and 8.42 days on red and green cultivars, respectively, Durations of pre-oviposition, oviposition and post-oviposition periods were not influenced by cultivars. Life span was 35.75 and 35.83 days on both cultivars respectively. Female fecundity did not vary significantly by 43.17 and 44.17 eggs/female (Table 4).

Table 3: Mean developmental time, and biological parameters (± SE) of *Phytoseiulus persimilis* reared on *Ricinus communis* (Red and Green cultivars).

Developmental stages	Host plant		T value
	Red cultivar	Green cultivar	
Egg	3.33 ± 0.14 a	3.50 ± 0.15 a	0.804 ns
Larva	1.00 ± 0.00 a	1.25 ± 0.13 a	1.915 ns
Protonymph	1.50 ± 0.15 a	1.67± 0.14 a	0.804
Deutonymph	1.42 ± 0.15 a	1.58 ± 0.15 a	0.793
Female life cycle	7.25 ± 0.18 b	8.00 ± 0.00 a	4.180**
Male life cycle	5.83 ± 0.21	6.17± 0.21	
Pre-oviposition period	1.00 ± 0.00 b	1.42 ± 0.15 a	2.803**
Oviposition period	27.67± 0.58 a	25.83 ± 0.65 b	2.103*
Post-oviposition period	1.92 ± 0.19 a	1.58 ± 0.29 a	0.962
Female longevity	30.58 ± 0.68 a	28.83 ± 0.59 a	1.948 ns
Male longevity	29.08 ± 0.67	26.33 ± 0.40	
Female lifespan	37.83 ± 0.73 a	36.83 ± 0.59 a	1.07 ns
Male lifespan	34.92 ± 0.61	32.50 ± 0.45	
Fecundity	53.42 ±1.10 a	48.25 ±1.05 b	3.398*
Egg/♀ / day	1.93 ± 0.02 a	1.87± 0.02 a	2.028 ns

The means in each row with the same letters are not significantly different (t-test, SPSS) ns= no significans, *p< 0.05; **P < 0.01.

The life table parameters listed in Table 5, for the predatory mites *P. persimilis* reflect a comparable values between the two cultivars (no significant difference). Values of *r*, λ and R_0 were (0.20, 1.22 and 26.63) (0.19, 1.21 and 23.88) for *P. persimilis* on red and green cultivars, respectively. For *N. californicus* were (0.19, 1.21 and 21.58) (0.19, 1.21 and 22.08) on red and green cultivars, respectively (Table 6).

Female age-specific life table lx and mx (Figure 4). The highest daily fecundity (*mx*) of *P. persimilis* occurred at the age 28 and mortality started on day 17 for both red and green cultivars. The highest daily fecundity (*mx*) of *N. californicus* was on the age 25 for the red cultivar, while it was on the age 20-25, 26 for the green cultivar. Mortality started on day 18 and 17 for red and green cultivars, respectively.

Table 4: Mean developmental time, and biological parameters (± SE) of *Neoseiulus californicus* reared on *Ricinus communis* (Red and Green cultivars).

Developmental stages	Host plant		T value
	Red cultivar	Green cultivar	
Egg	3.33 ± 0.14 a	3.50 ± 0.15 a	0.804
Larva	1.42 ± 0.15 a	1.33 ± 0.14 a	0.405
Protonymph	1.58 ± 0.15 b	2.00 ± 0.00 a	2.803**
Deutonymph	2.00 ± 0.00 a	1.58 ± 0.15 b	2.803**
Female life cycle	8.33 ± 0.14 a	8.42 ± 0.15 a	0.405 ns
Male life cycle	6.33 ± 0.14	6.08 ± 0.19	
Pre-oviposition period	1.50 ± 0.15 a	1.42 ± 0.15 a	0.394
Oviposition period	23.58 ± 0.72 a	24.08 ± 0.75 a	0.479 ns
Post-oviposition period	2.33 ± 0.38 a	1.92 ± 0.36 a	0.803
Female longevity	27.42 ± 0.65 a	27.42 ± 0.53 a	0.000 ns
Male longevity	24.50 ± 0.47	25.17 ± 0.42	
Female lifespan	35.75 ± 0.59 a	35.83 ± 0.52 a	0.106 ns
Male lifespan	30.83 ± 0.44 a	31.25 ± 0.35 a	
Fecundity	43.17±1.52 a	44.17 ± 1.16 a	0.523 ns
Egg/♀ / day	1.83 ± 0.03 a	1.84 ± 0.02 a	0.276

The means in each row with the same letters are not significantly different (t-test, SPSS)

ns= no significans, *p< 0.05; **P < 0.01.

Table 5: Life table parameters (mean ± SE) of *Phytoseiulus persimilis* reared on *Ricinus communis* (Red and Green cultivars).

Life table parameters	P. *persimilis*	
	Red cultivar	Green cultivar
Intrinsic rate of increase (*r*)	0.20±0.02 a	0.19±0.02 a
Finite rate of increase (*λ*)	1.22±0.02 a	1.21±0.02 a
Net reproductive rate (R_0)	26.63±5.46 a	23.88±4.90 a
Mean generation time (*T*)	16.23±0.38 a	16.88±0.31 a
Gross reproductive rate (*GRR*)	46.29±2.87 a	41.46±2.49 a

Data in each cultivar is related to the age-stage, two-sex life table. Mean values in a row followed by different letters are significantly different (Paired bootstrap test, P ≤ 0.05).

Table 6: Life table parameters (mean ± SE) of *Neoseiulus californicus* reared on *Ricinus communis* (Red and Green cultivars).

Life table parameters	N. *californicus*	
	Red cultivar	Geen cultivar
Intrinsic rate of increase (*r*)	0.19±0.02 a	0.19±0.02 a
Finite rate of increase (*λ*)	1.21±0.02 a	1.21±0.02 a
Net reproductive rate (R_0)	21.58±4.47 a	22.08±4.55 a
Mean generation time (*T*)	16.35±0.30 a	16.23±0.33 a
Gross reproductive rate (*GRR*)	32.96±2.79 a	34.99±2.62 b

Data in each cultivar is related to the age-stage, two-sex life table. Mean values in a row followed by different letters are significantly different (Paired bootstrap test, P ≤ 0.05).

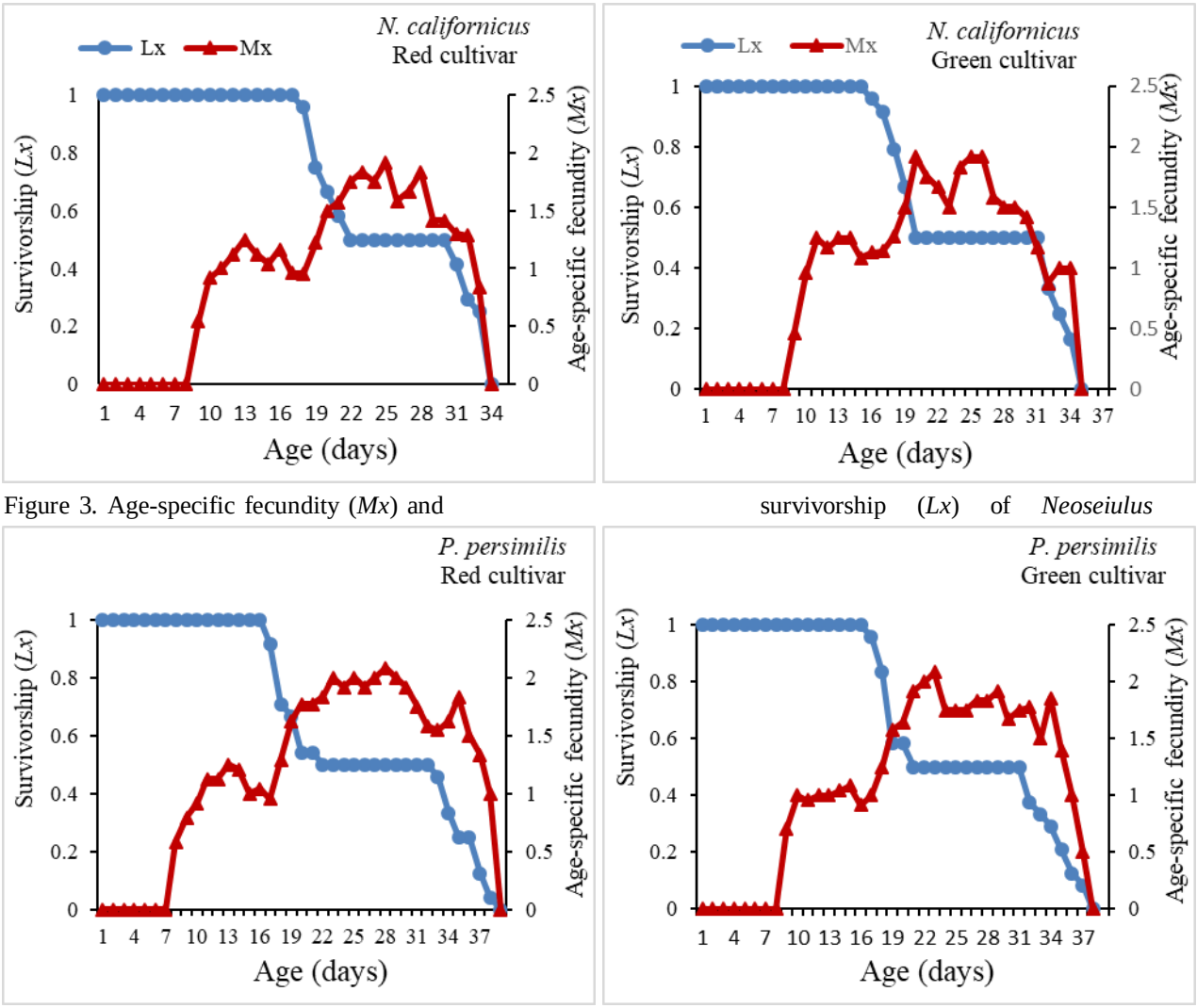

Figure 3. Age-specific fecundity (*Mx*) and survivorship (*Lx*) of *Neoseiulus californicus* and *Phytoseiulus persimilis* reared on *Ricinus communis* (Red and Green cultivars).

Discussion

The present study implied a comparison between two cultivars red and green of *R. communis* plant extracts. The total alkaloids in green leaves are more than red leaves which amounted to 0.088 and 0.066g/g powder crude extract for two types respectively. Ricinine is a natural insecticide from RC plant leaves. It was isolated and identified from chloroformic extract which is compatible with [32] that used the chloroform to extract and purification process of ricinine. Ricinine content was 0.533% in castor bean meal, Chen and Chen [32]. While, the herein percentage of predominant alkaloid i.e ricinine yield in RC leaves reached 0.59% in green leaves and 0.49% in red leaves. [12]. The purity of ricinine was over 98% (its retention time was 4.57 min in UPLC), while De Melo Cazal et al., [31] observed 96% purity of ricinine. The obtained results herein are consistent with those mentioned by De Melo Cazal et al., [31], Li et al [6] and Chen and Chen [32]. It is also worth mentioning that the relatively higher percentage of ricinine in green leaves than the red one may interpret the higher reproduction rates observed using red leaves.

The aim of this study was to evaluate the castor bean plant as an alternative host for *T. urticae* because of its lower cultivation cost besides its resistance to many biotic and abiotic stresses in predatory mite mass rearing process. Abd-Alla et al., [12] mentioned that the red cultivar has a huge amount of toxic secondary metabolites particularly ricinine in addition to the crude extract having an acaricidal effect. However, the present study showed that both red and green landraces with thicker leaves represented a natural trap for *T. urticae* in the inter-season periods. Red and green cultivars had significant effect on *T. urticae* developmental time and reproduction. Also, the effect was significant for *P. persimilis*, but not for *N. californicus*. The relationship between the host plant of *T. urticae* and the phytoseiid predators can be addressed in the following respects, the direct harmful effect due to secretion of the leaf glandular trichomes as tomatoes [6], or the phytochemicals of such medicinal plant castor bean e.g montoterpenes and phenols with their pesticidal or repellent effect. The responses of predatory mites to these volatiles are considered to be a crucial factor in the extermination of prey populations by phytoseiids such as *P. persimilis*. Prey-induced plant volatiles are highly detectable and can be reliable indicators of both prey presence and identity. Badawy *et al.*, [33] stated that the acaricidal activity of several monoterpenes from plant sources against *T. urticae*, was examined using fumigation and direct contact application methods. He mentioned that toxicity behavior of menthol, thymol, limonene etc. The results of Abd-Alla et al., [12] suggested that the natural product derived from the red cultivar RH21 of *R. communis* may have potential use as an acaricidal agent which may interpret the scarce natural infestation of red cultivar with *T. urticae* except that with thick leaves. However, it is too rare to find. The present study shows normal biology and reproduction rate for the prey *T. urticae* as well as the two predators. Comparison between reproduction parameters of *T. urticae* reared on castor bean in the present study are consistent with those of Abd-Alla et al., [12] that the red hendi cultivar was more appropriate for *T. urticae* hence female fecundity reached 73 eggs/female on red cultivar with only 55 eggs on green one. In our study, the average numbers of egg per day was 4.71 eggs / day on the red cultivar with only 3.77 eggs / day on the green one. When *T. urticae* was reared on mulberry, sweet potato and castor bean the shortest durations of longevity and life span were recorded on castor bean leaves (8.5 and 20.26 days, respectively), the average numbers of eggs /day when *T. urticae* was reared on castor bean leaves was 4.45 eggs/day [34]. The rearing on both red and green cultivars has been slightly increased for *P. persimilis* fecundity by laying 53.4 and 48.5 eggs/female on both cultivars respectively.

Conclusions

The conclusion hypothesis of our results is that the RC plant leaves were used as an alternative host for *T. urtica* because of the relatively higher percentage of ricinine in green leaves than the red ones and this explains the higher reproduction rates observed using the red leaves. Its potent lower cultivation cost besides resistance to many biotic and abiotic stresses in predatory mite mass-rearing process. Both red and green cultivars represented a natural trap for *T. urticae* in the inter-season periods. Feeding of *T. urticae* on red and green cultivars in the laboratory did not have a significant antagonistic effect neither on it nor feeding of *P. persimilis* and *N.californicus.*

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