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Research Article

Effects of Three Neonicotinoid Insecticides on the Development and Fecundity of Red Spider Mite: An age-stage two sex life table study

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ABSTRACT

Red spider mite, *Tetranychus urticae* Koch, causes severe damage to economically important crops, fruits, vegetables, ornamentals etc. Management of red spider mite largely depends on chemicals. Application of insecticides may cause massive outbreaks of mite population. Sublethal effects of imidacloprid, thiamethoxam and acetamiprid doses were investigated on life-table parameters of red spider mite. All neonicotinoids revealed significant effects on life characteristics i.e. development duration and fecundity of red spider mite. Mites treated with imidacloprid, thiamethoxam and acetamiprid significantly reduced the net reproductive rate (R_o). No significant difference in finite rates of increase (λ) was observed among the treatments. Imidacloprid reduces the egg duration and mean generation time (T). Emerged adult longevity was shorter when exposed to insecticides. The total adult longevity was significantly reduced by the insecticides in comparison with the control. Highest mean generation time was observed in acetamiprid treated mites. The results suggested that sublethal effects of tested insecticides affected the biological parameters of red spider mite.

Keywords: Imidacloprid; life table; sublethal effects; *Tetranychus urticae*; thiamethoxam

INTRODUCTION

The red spider mite (RSM), *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), is one of the most economically important polyphagous pest worldwide [1, 2, 3]. It is the generalist spider mite species and colonizes about 1,100 known plants species belonging to 140 plant families [4, 5]. It damages plants by inserting its stylet into plant epidermis, sucking the cell sap which causes cell death, defoliation, plant death and causes reduction in yield [6, 7]. It is often very difficult to control RSM because of its small size, short biological cycle, parthenogenesis, abundant progeny and propensity, which contributes to develop capacity to resist to various classes of insecticides [8, 9, 10].

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The life cycle consists of the following stages: the egg, larva, protonymph and deutonymph, and adult. Temperature has a major impact on how long it takes an egg to become an adult. Red spider mites need five to twenty days to finish developing at optimal temperatures (about 80ºF). Every year, there are numerous overlapping generations.

Like other countries, in Pakistan prevention and management of RSM is mainly based on chemical pesticides. Repetitive and excessive use of the pesticides resulted in RSM resurgence, high-level resistance development and destroys natural enemies' population which made this type of pest more challenging to control [11, 12, 13, 14]. About 501 resistance cases for 95 active ingredients in RSM have been reported and it is considered as "Resistant Champion" and it causes its control more problematic [15].

Neonicotinoids are the most rapidly growing, frequently and commonly used class of pesticides [16]. These insecticides were registered in 120 countries for the management of large number of polyphagous pests. These highly specific insecticides disrupt the nicotinic acetylcholine function in insects [16, 17]. Initially their success is due to their safety profile to non-target organisms, their biological and chemical properties as well as excellent systematic characteristics [16]. However, frequent application of these insecticides has negative environmental effects and result in severe outbreaks of many species of family Tetranychidae on the wide range of crops, shrubs and trees [14, 18,]. The enormous outbreaks of RSM are potentially due to increase in the reproduction of mite by imidacloprid along with suppression of biological control agents [19]. Also, acetamiprid came out as higher tendency for the population's buildup of tetranychid mite.

Lethal and sublethal doses of some pesticides may be source of increase in the population of the mite. So, it is imperative to understand the effects of lethal and sublethal doses and risk associated with pesticide applications [14]. Considerable research has reported the lethal and sublethal effects of pesticides on growth parameters of RSM [20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30]. Studies regarding sublethal effects comprise of negative effects on the population except mortality, including reduced longevity, reduced fecundity, increased developmental time, and changes in the sex ratios [23, 28, 30, 31, 32, 33]. It gives comprehensive knowledge of total effects of pesticides, and it is highly recommended for the evaluation of lethal and sublethal implications of pesticides on demographic parameters [21, 27, 34, 35].

The study explores the stimulatory effect of field recommended and sublethal doses of three commonly used neonicotinoid insecticides including thiamethoxam, acetamiprid and imidacloprid on life table parameters of RSM.

MATERIAL AND METHODS

RSM and Insecticides

This study was conducted during 2020-2021 at laboratory of University of Agriculture, Faisalabad, Pakistan. Mites were collected from infested leaves of the Bakain (*Melia azedarach*) from University of Agriculture, Faisalabad, Pakistan. Mites were reared on *M. azedarach* in laboratory-controlled conditions (RH 70±5% and Temperature 28°C ± 2°C) for at least three generations before using them for experiments. The same laboratory conditions were used for bioassays studies.

The Insecticides used in the study namely: Thiamethoxam (Actara 25 WG), Imidacloprid (Crown 25% WP) and Acetamiprid (Assault 20% SL) were tested at field recommended doses (95,100, 125 ppm) and sublethal doses (9.5, 10, 12.5ppm) respectively.

Life-table bioassays

Effects of lethal and sublethal doses of neonicotinoids were investigated on life-table growth parameters of RSM. For each treatment thirty (30) RSM females (one day old) were used. Eggs laid by adult females were transferred to rearing cells using a fine camel hairbrush. Individual leaf discs treated with test insecticides by dipping leaves in a solution for 5s and dried at room temperature were placed in rearing cells. Leaf discs treated with distilled water used as a control. Leaf discs were kept moist and changed when necessary, during the assays. Rearing cells were observed under binocular microscope. Observations were recorded at 24h intervals during study. Development of the Egg development was observed on daily basis. 30 newly hatched nymphs were selected and then shifted to a freshly treated leaf disc with one nymph per leaf disc. Daily nymph development was recorded. During observation different aspects of biology and life history of RSM like eggs hatching period, larval, protonymphal, deutonymphal duration and adult stage were recorded. One RSM female and one male were introduced for mating and male adults were removed after 48h. The fecundity of RSM was recorded daily. Laid eggs were removed daily from the cells after counting. The experiment was continued until all adult females died and escaped females were excluded from the analysis.

Data Analysis

Data regarding the survivorship, longevity and fecundity was analyzed using computer program TWOSEX-MS Chart; according to age-stage, two-sex lifetable [36]. The effects of lethal and sublethal doses of neonicotinoids on the survival and development of RSM, adult longevity were assessed by analyses of variance (ANOVAs) with Statistix 8.1 Software. Means were compared by Tukey's HSD ($P < 0.05$), when ANOVA was significant. Life table attributes, such as reproduction rate (R_0), intrinsic rate of increase (r), mean generation time (T), finite rate of increase (λ), Gross reproductive rate (GRR), net age-stage specific survival rate (S_{xj}), age-specific survival rate (l_x), age-stage specific fecundity (f_x), age-specific fecundity (m_x), age-specific maternity ($l_x m_x$), age-stage life expectancy (e_{xj}) and age-stage-specific reproductive value (v_{xj}) were calculated [37, 38]. The Bootstrap method (100,000 replications) was used to estimate means and standard errors were estimated [39]. Tukey's HSD was conducted for comparison of means among the treatments. GraphPad Prism 8.0 was used for Plots preparation for survival, fecundity, maternity, life expectancy, and reproductive value.

RESULTS AND DISCUSSION

Much work on the systematic and biology of phytophagous mites has been done in the world as well as in Pakistan, but enough work has not yet been reported on massive out breaks of RSM followed by application of different insecticides.

A number of studies were conducted to study the effect of lethal and sublethal doses of different insecticides groups like organophosphates, pyrethroids, tetrazines, azomethine, pyridines and neonicotinoids on the demographic parameters of RSM. Researchers found an increase in the population of mites by using insecticides rather than reducing the population of the RSM [26, 40, 41, 42, 43, 44]. Due to physiological and biochemical adaptation, a potential increase in the population was found rather than the suppression after the insecticide's application [26, 44, 45].

The effects of recommended and sublethal doses of neonicotinoid insecticides (Thiamethoxam, Imidacloprid and Acetamiprid) on the development of RSM are summarized in Table 1.

Table 1. Development parameters (mean ± SE) of red spider mite on various doses of neonicotinoid insecticides

Parameters	Thiomethoxam		Imidacloprid		Acetamiprid		Control
	Recommended Dose	Sublethal Dose	Recommended Dose	Sublethal Dose	Recommended Dose	Sublethal Dose	
Egg duration (Days)	1.73 ± 0.08 [c]	2.00 ± 0.09[c]	2.00± 0.11[c]	1.73 ± 0.08[c]	5.30 ± 0.12[a]	3.33 ± 0.08[b]	3.36 ±0.10[b]
Larval duration (Days)	2.00 ± 0.15[c]	2.30 ± 0.10[c]	2.00 ± 0.12[c]	2.00 ± 0.10[c]	4.66 ± 0.15[b]	6.70 ± 0.12 [a]	2.33 ± 0.08[c]
Protonymph duration (Days)	1.60 ± 0.19[de]	2.00 ±0.17[cd]	2.30 ±0.22[cd]	1.00 ± 0.10[e]	3.60 ±0.36[a]	3.30 ±0.29[ab]	2.6 ± 0.14[bc]
Deutonymph duration (Days)	1.00 ± 0.17[c]	1.30 ±0.13[bc]	2.30 ± 0.30[a]	1.00 ± 0.11[c]	2.00 ± 0.35[ab]	1.33 ± 0.18[bc]	2.33 ± 0.19[a]
Adult Longevity (Days)	3.80 ± 0.24[e]	5.00 ± 0.12[d]	8.90 ± 0.32[b]	6.17 ± 0.16[c]	3.30 ± 0.23[e]	3.20 ± 0.20[e]	12.7 ±0.21[a]

Developmental duration of the different stages of RSM is affected by the insecticides. The developmental time of the egg stages was shortened in thiamethoxam and imidacloprid concentrations as compared to control (F = 333, df = 6, P < 0.01). Acetamiprid at recommended dose prolonged the duration of egg stage. At larval stage, no significant difference was observed on the development period of imidacloprid and thiamethoxam treated mites (Table 1).

Untreated mites protonymph duration was 2.6±0.14 days and it significantly reduced by thiamethoxam and imidacloprid doses, but acetamiprid prolonged the duration of protonymph (F = 24.4, df = 6, P < 0.01). Deutonymph durations were significantly shortened as compared to control when exposed to sublethal doses of thiamethoxam, acetamiprid and imidacloprid.

Emerged adult longevity was shorter when exposed to insecticides. The total adult longevity was significantly reduced by the insecticides in comparison with the control (F = 247, df = 6, P < 0.01). Among the insecticides, acetamiprid exposed mites lived for the shortest duration. Mean total pre-adult developmental times were significantly different from control at all stages when mites are treated with acetamiprid doses. Acetamiprid doses had significant toxic effect on RSM adults and the number of eggs/ emale was reduced by 55.2% and longevity 70.08% as compared with control group (Table 1). Our findings agree with Ako*et al*. (2004) which investigates the effect of four neonicotinoid insecticides i.e thiamethoxam (25 WG), imidacloprid (200 SL), acetamiprid (70 WP) and thiacloprid (480 SC) on fecundity of RSM.

A significant decrease in adult longevity of the adult by 60.6%, 74.80%, 51.41% was observed after treatment of thiamethoxam, imidacloprid and acetamiprid sublethal doses respectively as compared with the control group. Similarly, sublethal doses of spinetoram, diflovidazin and fenazaquin have been shown to reduce the longevity by 20.07%, 23.90% and 31.71% respectively. Significant reduction in longevity observed by sublethal doses of spirodiclofen, abamectin and pyridaben. Spirotetramat also reduced longevity in dose dependent manner [23, 26, 28, 29]. It is found that sublethal concentrations of fluralaner (LC_{30}), chlorfenapyr (LC_{30}) and diflovidazin (LC_{20}) could decrease the adult longevity [28, 30]. Reduction in adult female duration was recorded by exposure of sublethal concentrations of bifenthrin (LC_{25}) and spinetoram (LC_{20}) [23, 26]. Clofentezine treatment also reduced the longevity and fecundity of survival females [31].

According to S_{xj} (age-stage specific survival rate) of RSM on different concentrations of insecticides, the probability of survival of a newly borne individual to age *x* and stage *j* were shown in Fig 1. A detectable overlap in these curves was observed at different developmental stages between the individuals in all treatments. The highest peak for survival of female was observed in control and highest male peak found in thiamethoxam sublethal dose.

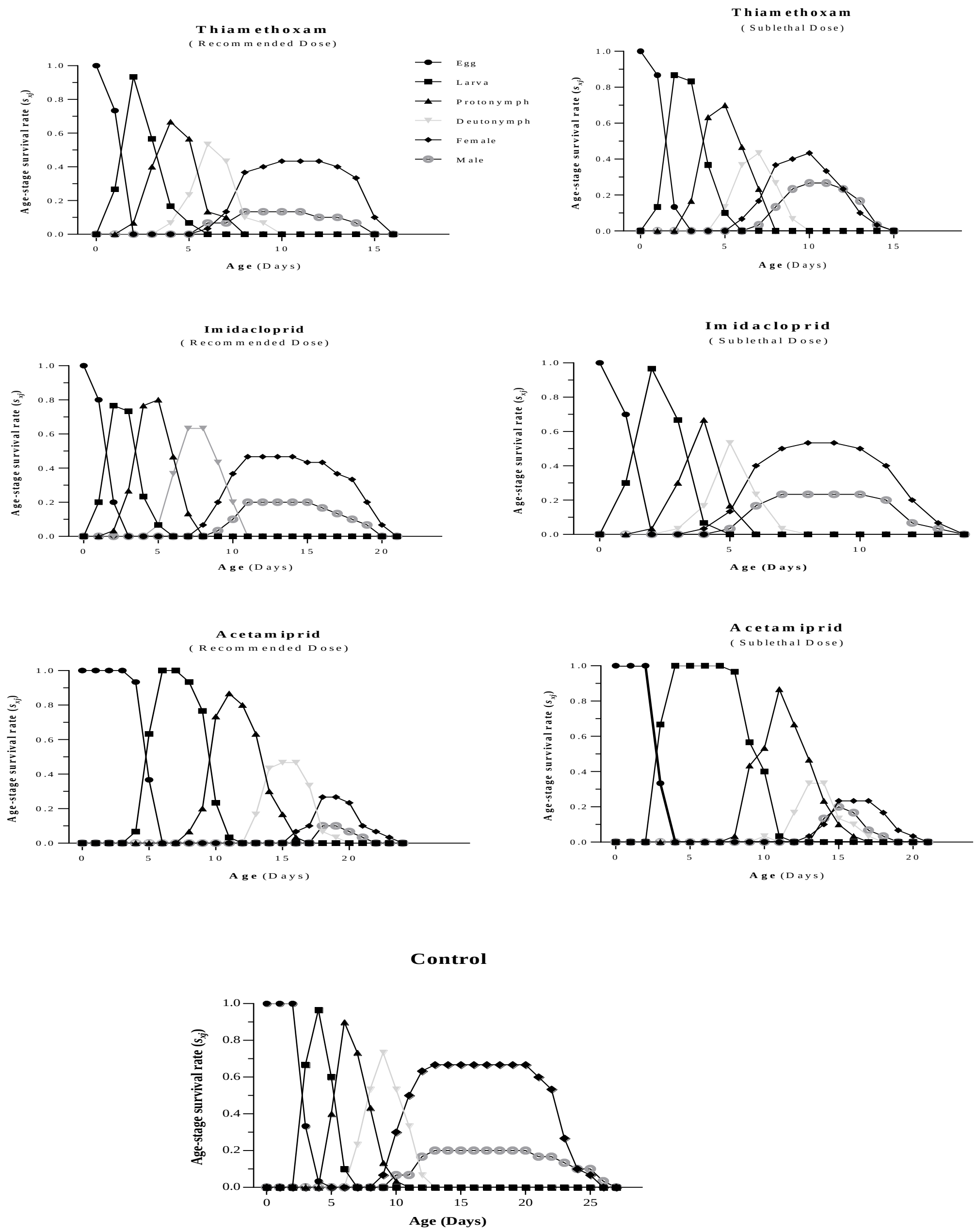

Figure 1. Age-stage specific survival rate (S_{xj}) of red spider miteafter treated with different doses of neonicotinoid insecticides.

The age-specific survival rate (l_x) was evaluated. It is the probability of an egg of RSM which will survive to age *x*, and the curve for the l_x is calculated by integrating all surviving individuals regardless of developmental stage (Fig 2). The lowest survival curve of all age stages was observed in RSM treated with imidacloprid sublethal dose. According to l_x, the total life span of untreated RSM was 27 days but it was 15 and 16 days when RSM are treated with thiamethoxam recommended and sublethal concentrations respectively.

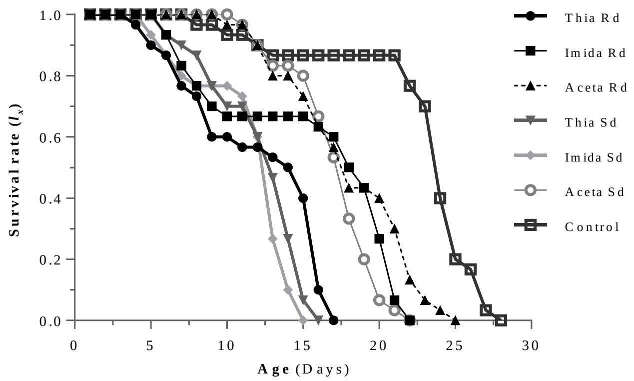

Figure 2. Age-specific survival rate (l_x) of red spider mitetreated with different doses of neonicotinoid insecticides compared with control.

(Thia Rd= Thiamethoxam recommended dose; Imida Rd=Imidacloprid recommended dose; Aceta Rd= Acetamiprid recommended dose; ThiaSd= Thiamethoxam sublethal dose; ImidaSd=Imidacloprid sublethal dose; AcetaSd= Acetamiprid sublethal dose)

When RSM eggs were treated with neonicotinoid insecticides, an unexpected decrease in duration of eggs was observed by the imidacloprid and thiamethoxam. However, acetamiprid at field recommended dose increases the egg duration (Table 1). An increase in the egg duration by fluralaner (LC_{30}) and bifenthrin respectively was reported [23, 30]. Larval duration was increased by acetamiprid up to 65.23%. Our findings are in accordance with Wang *et al* 2014 report.

Fig 3 and 4 compare the *fx* (age- specific fecundity) and m_x (maternity) of individuals in all the treatments. Peaks of *fx* and m_x were higher in control in comparison with other treatments. The lowest peaks of *fx* and m_x were observed in recommended doses of thiamethoxam and acetamiprid treated RSM. On 17th day of untreated RSM life span, the highest value of m_x (8.34 eggs/individual/day) was recorded. When RSM treated with imidacloprid recommended and sublethal dose, m_x value was approximated to be 4.80 and 4.08 eggs/individual/day occurred on 17th and 8th day respectively (Fig 4). The lowest higher m_x value was recorded in thiamethoxam treated RSM. Untreated RSM showed maximum $l_x m_x$ value at seventeen days. But the minimum $l_x m_x$ was observed in thiamethoxam treated mites (Fig 5).

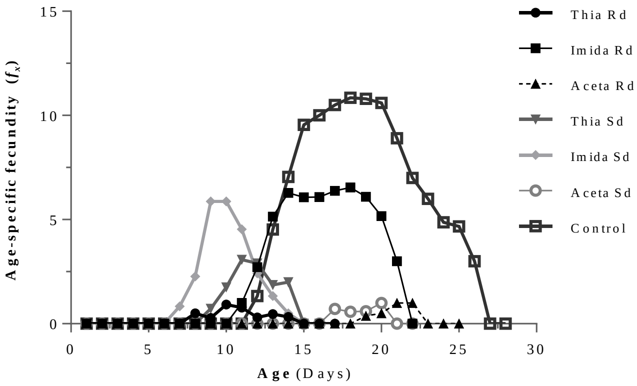

Figure 3. Age-specific fecundity (f_x) of red spider mitetreated with different doses of neonicotinoid insecticides compared with control

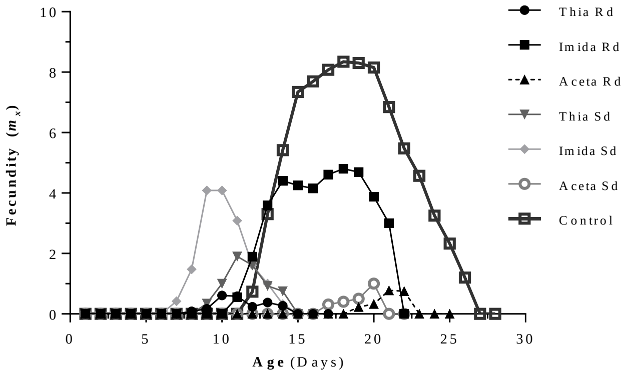

Figure 4. Age-specific fecundity of total population (m_x) of red spider mitetreated with different doses of neonicotinoid insecticides compared with control.

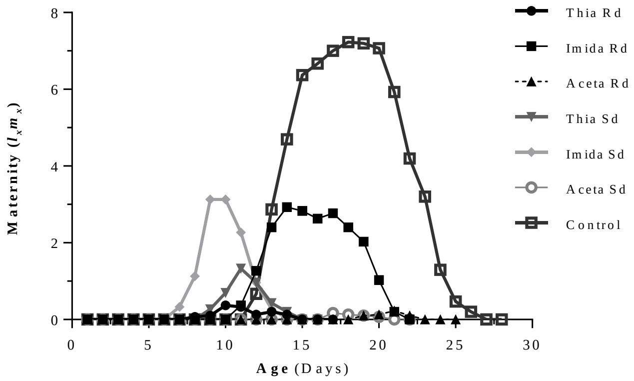

Figure 5. Age-specifc maternity ($l_x m_x$) of red spider mitetreated with different doses of neonicotinoid insecticides compared with control.

Fecundity was significantly reduced by all the insecticides at field relevant doses in comparison with the control while no significance was observed among the sublethal doses of the insecticides. A 2.8-3.5-fold reduction in the fecundity by thiamethoxam was also reported after foliar residual exposure and drench application [42]. Systematic applications of thiamethoxam and imidacloprid did not increase the fecundity of RSM [43]. Number of eggs/females was reduced by 33.45% and longevity by 22.45% when RSM treated with sublethal dose (LC_{30}) of fluralaner as compared to control [30]. Despite that, James and Price (2002) [40] reported that drench and foliar application of imidacloprid significantly increased the fecundity of RSM. Similar observations were described by Castagnoli *et al*. (2005) [46] about the increase of fecundity of adult mites. Under laboratory conditions, thiamethoxam treatment also slightly increases the fecundity of RSM [43]. Number of eggs laid by female was significant increase when treated with LC_{20} of spinetoram which results in outbreaks of mite population [26]. Similar results of other published reports have been observed regarding the effects of acaricides on mites. [26, 28] For example, 23.23% reduction in number of eggs per female was observed by LC_{25} of bifenthrin [23].

The parameter age-stage-specific expectancy of life (e_{xj}) is the duration of expected survival of an individual of age *x* and stage *j*. E_{xj} of RSM each stage was plotted in Fig 6 and were affected by insecticide applications. The life expectancy of thiamethoxam treated individual was shorter and the longest was observed in untreated mites.

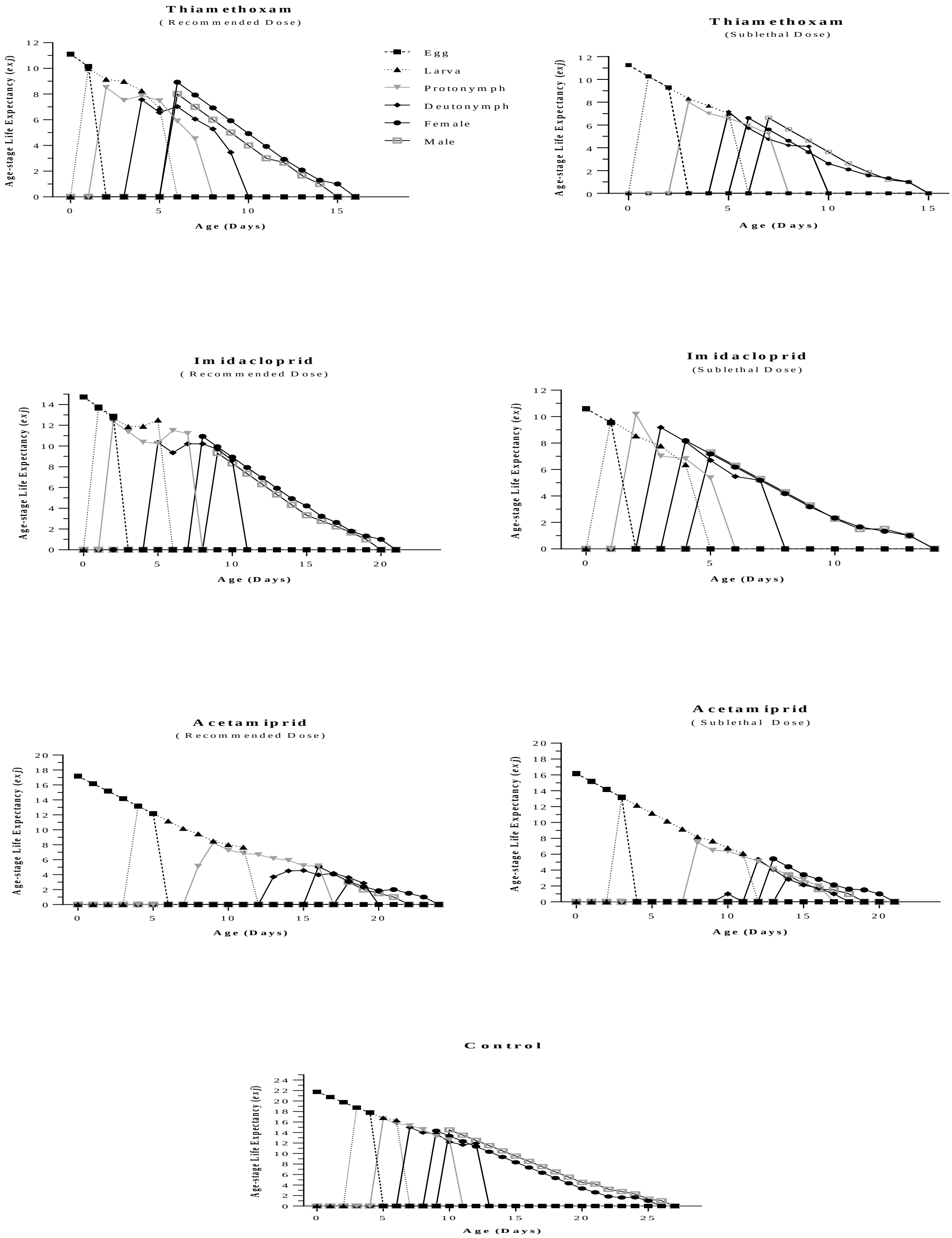

Figure 6. Age-specifc life expectancy (e_x) of red spider mite treated with different doses of neonicotinoid insecticides compared with control

Age-stage-specific reproductive value (v_{xj}) is the prediction of the future population by an individual contribution at age x and stage j. Significant reductions in the curves of reproductive value were found in insecticide treated RSM (Fig 7). The peak reproductive value was recorded in control at the age of 10.

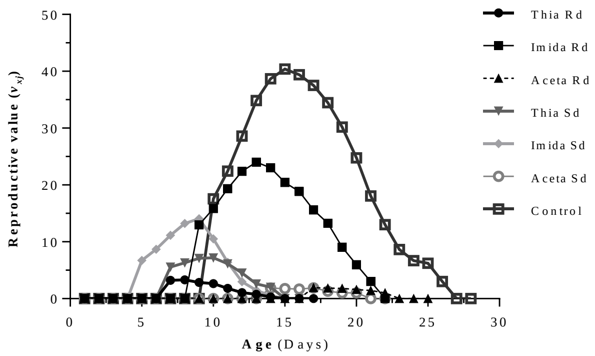

Figure 7. Age reproductive (v_{xj}) of red spider mite treated with different doses of neonicotinoid insecticides compared with control.

Results regarding the population growth parameters were presented in Table 2. Acetamiprid treatments reduced the net reproductive rate (R_0) of RSM (0.4677 ± 0.180) in comparison with the control (65.06 ± 8.541), significantly. Imidacloprid and thiamethoxam doses as well had significantly lower R_0. In Acetamiprid and thiamethoxam treatments, *the r value* was reduced in compare with control (Table 2). No significant difference in finite rates of increase (λ) in population was observed among the different treatments. Mean generation time (*T*) was also significantly reduced in imidacloprid and thiamethoxam treated RSM. Gross reproductive rates (*GRR*) were significantly affected by the insecticides (Table 2).

Table 2. Population growth parameters (mean ± SE) of red spider miteon different doses of neonicotinoid insecticides

Population Parameters	**Thiomethoxam**		**Imidacloprid**		**Acetamiprid**		**Control**
	Recommended Dose	Sublethal Dose	Recommended Dose	Sublethal Dose	Recommended Dose	Sublethal Dose	
***r* (d^{-1})**	0.025 ± 0.032^{d}	0.121± 0.021^{c}	0.199± 0.145^{b}	0.250± 0.020^{a}	-0.027± 0.027^{e}	-0.041± 0.024^{f}	0.244 ±0.0089^{a}
λ (d^{-1})	1.026 ± 0.033^{a}	1.129± 0.023^{a}	1.220 ± 0.176^{a}	1.284± 0.025^{a}	0.972 ± 0.025^{a}	0.95 ± 0.022^{a}	1.276± 0.0113^{a}
***T* (days)**	11.09 ± 1.795^{de}	11.21 ±0.304^{d}	15.26 ±0.282^{c}	9.67± 0.24^{e}	20.601 ±2.346^{a}	18.167 ±0.911^{b}	17.06± 0.236^{b}
R_0 (offspring/individual)	1.33 ± 0.436^{de}	3.9 ± 0.853^{d}	20.86 ± 4.25^{b}	11.26 ± 1.95^{c}	0.566 ± 0.263^{e}	0.4677± 0.180^{e}	65.06± 8.541^{a}
***GRR* (offspring/individual)**	2.33 ± 0.673^{e}	6.54 ± 1.427^{d}	39.84 ± 5.69^{b}	16.1 ± 2.37^{c}	2.09 ± 0.935^{de}	2.21± 0.48^{e}	81.07± 9.643^{a}

According to the results shown in Table 2, neonicotinoids affects most of the life table growth parameters of RSM. A *r* (increase in intrinsic rate) decrease in population leads to adverse effect, resulted in fewer offsprings were observed in all treatments as compare to untreated except in sublethal dose of imidacloprid. It is the best population parameter which indicates the comprehensive effect of pesticides on both survival and fecundity of the population [27, 47]. results with clofentezine and diflovidazin treatment on RSM was observed by Marcic 2003 and Havasi *et al*. 2018 [28, 31]. Also, Bifenthrin sublethal concentrations (LC_{10}, LC_{25}) concentrations significantly decreased the intrinsic rate of increase (*r*) [23]. While an increase in *r* in spinetoram treatment was also observed [26]. In our study, no obvious differences were noticed in finite rate of increase (λ) among various doses of treatments. The results are in congruent with Sáenz-de-Cabezón *et al*. (2006) [48] and Havasi *et al*. 2018 [28], who studied the triflumuron and diflovidazin effect on RSM respectively. On the contrary, Wang *et al* 2014 [23] found reduction in the λ (finite rate of increase). Furthermore, the highest net reproductive rate (R_0) belongs to control and significant reduction was observed in other treatments. Ourfindings in the present study are in consistent with Marcic (2007) [49], Wang *et al*. (2014) [23], Havasi *et al*. (2019) [50] as they studied the sublethal effects of spirodiclofen, bifenthrin, diflovidazin and thiamethoxam respectively. Marcic *et al*. (2003) [31] also observed 2.6 times reduction in R_0 by the survival females with clofentezine. While in contrast, Barati *et al*. [25] found the highest R_0 in thiacloprid and thiamethoxam treatments. Acetamiprid treated mites had highest mean generation time (*T*) and lowest *r*. Our results are in contrast with Barati *et al*. (2015) [25] which also observed significant reduction in generation time when treated with acetamiprid. Acaricides significantly influenced the mean generation time (*T*) of RSM.

The current study clearly demonstrates that serious effects of lethal and sublethal exposure of neonicotinoids on the population parameters of red spider mite like survival, fecundity, longevity, mean generation time, intrinsic rate of increase, and net reproductive rate though no significant on finite rate of increase. More research is a requirement in unveiling the sublethal effects of neonicotinoids for one or two generations. Based on our results, neonicotinoids can be used in IPM programs with necessary precautions.

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