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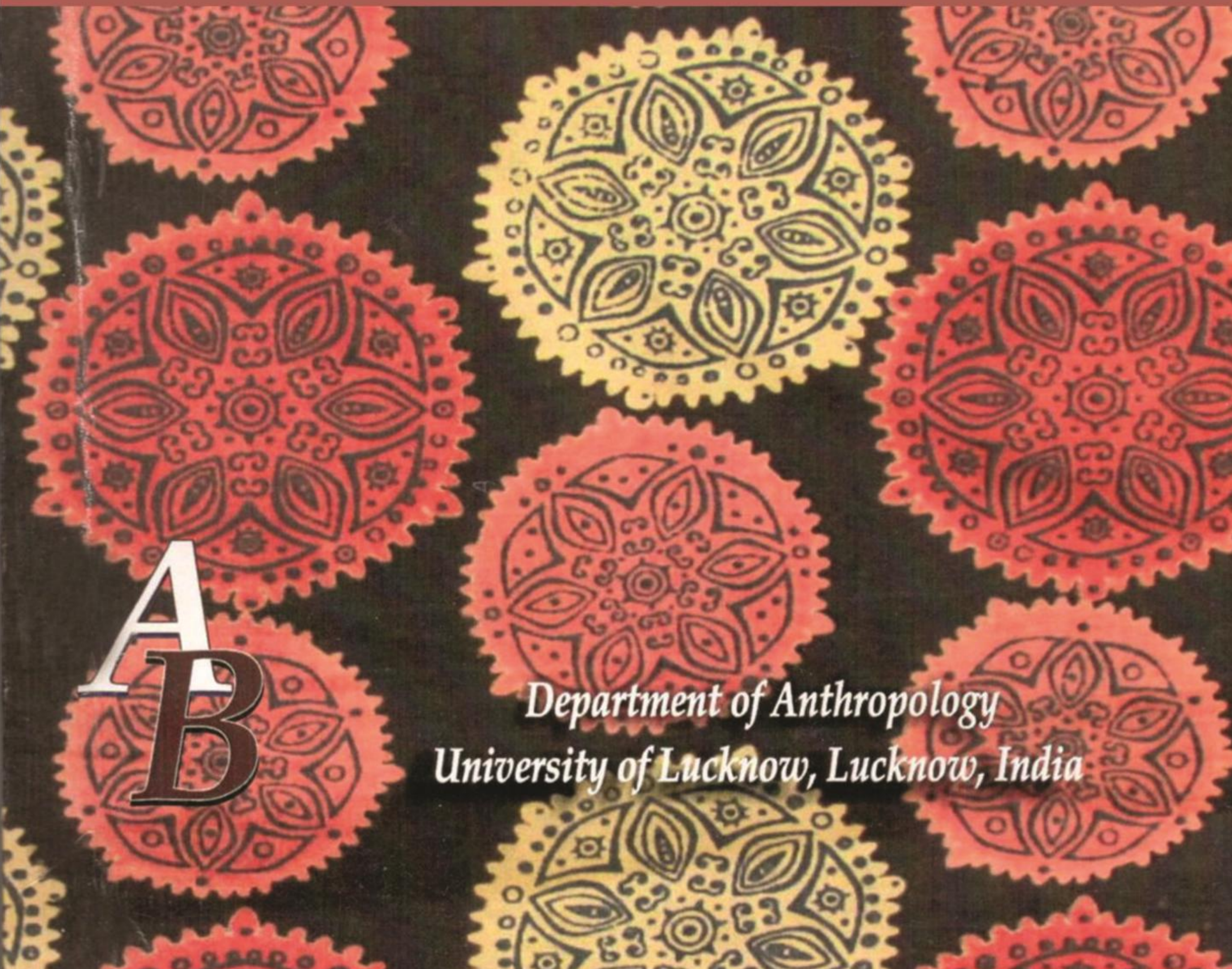

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Influence of temperature on the efficacy of imidacloprid against Red Cotton Stainer *Dysdercus koenigii* (Fabricius)

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ABSTRACT

In the present work, the insecticidal efficacy of topically applied sublethal concentrations (0.008%, 0.006%, 0.004%, 0.002%, and 0.001%) of imidacloprid on 24 hrs. old 4th instar nymphs of red cotton stainer (Dysdercus koenigii) at three different temperatures i.e., 30 ± 1ºC, 25 ± 1ºC and 20 ± 1ºC with constant relative humidity (60-70%) and photoperiod regime (14L:10D hrs.) were studied in the laboratory. The percentage of initial nymphal mortality within 24-hour, total nymphal mortality up to adult emergence and malformation of the treated nymphs increased linearly with increasing sublethal concentrations and shows positive correlation at all the three respective temperature and photoperiod. On the other hand, the fecundity and fertility of the adult female emerging from the treated nymphs decreased linearly with increasing sublethal concentrations and showed negative correlation. The toxicity in term of knockdown effect within 24-hour as well as residual effect up to adult emergence was comparatively higher at 30 ± 1ºC as compared to the 25 ± 1ºC and 20 ± 1ºC temperature, whereas deformities in wings as well as twisting of legs was more at 20 ± 1ºC temperature as compared to 25 ± 1ºC and 30 ± 1ºC temperature. Similarly, reduction in average egg production and average egg hatching were more pronounced at 20±1ºC as compared to the 25 ± 1ºC and 30 ± 1ºC temperature and photoperiod.

Keywords: Imidacloprid, *Dysdercus koenigii*, temperature, mortality, malformation, fertility, fecundity,

INTRODUCTION

Climate change is recognized as one of the most serious global challenges in the twenty first century (Bulkeley, 2013). It is mainly produced by anthropogenic activities which resulted significant risks to natural systems (National Research Council, 2011) that affect human health in many ways, including livelihood and food security (Ampadu et al., 2018; Antwi-Agyei et al., 2014). Climate variables such as change in temperature, change is rain fall, relative humidity could directly influence the action of insecticides use in insect pest management. The influence of temperature on the toxicity of insecticides has been documented by several workers like Reichenbach and Collins (1984), Alexandrescu (1986),

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Grafius (1986), Turnbull and Harris (1986), Morytz, et al. (1997), Cho, et al. (1999), Rahman, et al. (1999), Amarasekare and Edelson (2004), Musser and Shelton (2005). In some case the effectiveness of an insecticide may increase but in others it may decrease or may remain constant with changes in temperature. Several workers like Coats et al., (1989), Valles et al., (1998), Hodjati and Curtis (1999), Hardwood et al., (2009) have shown a decrease in insecticides toxicity with increasing in temperature, while, other have reported a positive correlation between temperature and insecticides toxicity. For example, alphamethrin and deltamethrin produced higher mortality in nymphs of clear-winged (*Camnula pellucida*) and two-striped (*Melanoplus bivittatus*) grasshoppers at higher temperatures (Johnson, 1990). The effectiveness of insecticides at different temperature also depends upon insect species. Li et al. (2006) observed a positive correlation between temperature and deltamethrin toxicity in armyworm and tobacco budworm but a negative correlation in cabbage looper. Increasing in temperatures are generally known to enhance the toxicity of pesticides as well (Lydy et al.,1999). Boina et al. (2009) studied the influence of posttreatment temperature (range, 17-37^{o}C) on the toxicity of selected organophosphate (chlorpyrifos and dimethoate), carbamate (carbaryl), avermectin (abamectin), pyrethroid (bifenthrin, zeta-cypermethrin, fenpropathrin, and lambda-cyhalothrin), and neonicotinoid (acetamiprid, imidacloprid, and thiamethoxam) insecticides against adult of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) and observed that all the tested organophosphates, carbamate, avermectin and neonicotinoids showed a positive temperature correlation within the 17-37^{o}C range, however with the exception of bifenthrin, all pyrethroids tested exhibited a negative correlation over the temperature range tested. Wadleigh et al. (1991) tested 10 pyrethroids on male german cockroaches *Blattella germanica* and observed that at LC50, the toxicity was negatively related with temperature. Thus, in the present investigation, the influence of temperature on the efficacy of imidacloprid against Red Cotton Stainer *Dysdercus koenigii* (Fabricius) have been studied under laboratory conditions at three different temperatures.

The red cotton stainer, *Dysdercus koenigii* (Fabricius), has emerged as an important major insect pest of cotton (Gossypium hirsutum) Linnaeus (Malvales: Malvaceae) worldwide from the last few years (Shah, 2014). This bug is found in southern Asia has become a major threat to cotton in India and Pakistan (Shahid et al., 2017). It is polyphagous nature it is found on number of crops but cotton is the most preferred host. Both adults and nymphs suck the sap from the emerging bolls and damage the lint quality by staining thus indirectly reducing the yield (Khan and Qamar 2012; Jaleel et al. 2014).

Imidacloprid, a neonicotinoid insecticide, is widely sprayed either alone or mixed with other insecticides against large number of insect pest. It was introduced in 1992 for the control of sucking pests. It is also used as foliar application and for seed treatment. It has a broad safety margin in mammals because of its lower affinity to the nicotinic receptors of vertebrates than those of insects.

MATERIAL AND METHODS

Adults of *D. koenigii* were obtained from the culture maintained at section of Entomology, Department of Zoology, Aligarh Muslim University, Aligarh. These were then reared in the laboratory in glass jars containing moist soil and were fed on cotton seeds (soaked overnight in water). The cultures were maintained at three temperatures viz. 30 ± 1^{o}C, 25 ± 1^{o}C and 20 ± 1^{o}C in different B.O.D incubators whereas the relative humidity and photoperiod regime were kept constant in each incubator (60-70% R.H and 14:10 hrs, L:D photo period). Preliminary studies were conducted to find out the LD_{50} value at each temperature. Based on LD_{50} value, five serially diluted concentrations viz., 0.008, 0.006, 0.004, 0.002 and 0.001 percent of insecticides were considered as the sublethal concentrations. For each temperature, one hundred newly moulted 24 hrs old 4th instar nymphs in four equal replicates were treated topically individually with 1µl sublethal concentrations of imidacloprid with 26-guage needle attached to a tuberculin syringe fitted into a manually operated applicator. A control group having the same number of nymphs was exposed to acetone only and regarded as control-I. Another control group having the same number of untreated nymphs was also maintained to compare acetone exposed insects and regarded Control-II. The effects were observed on mortality, malformation, fecundity, and fertility by using five sub lethal concentrations of insecticide at different temperature.

RESULTS AND DISCUSSION

In the present investigation the topical application of sublethal concentration of imidacloprid (viz., 0.008, 0.006, 0.004, 0.002, and 0.001%) on 24 hrs. old 4th instar nymphs of *Dysdercus koenigii* at three different temperatures (viz., 30 ± 1ºC, 25 ± 1ºC and 20 ± 1ºC) caused immediate mortality within 24 hrs. and successive mortality up to adult emergence. This initial mortality within 24 hours as well as mortality up to adult emergence increased with increasing sublethal concentrations at both the temperature and photoperiod. The regression between concentration strength and nymphal mortality within 24 hours at 30 ± 1ºC ($Y=3908.5x+18.38$, $R^2=0.958$, Fig.01), at 25 ± 1ºC ($Y=2914.6x+13.15$, $R^2=0.977$, Fig.01) and at 20 ± 1ºC ($Y = 3024.4x + 6.097$, $R^2 = 0.990$, Fig.01) yield a positive correlation. The initial nymphal loss within 24 hours after the topical application of highest selected sublethal concentration (i e 0.008%) at 30 ± 1ºC was 31% which was 07% and 14% more as compared to 25 ± 1ºC and 20 ± 1ºC temperature respectively. However, the topical application of lowest sublethal concentration (i.e., 0.001) at 30 ± 1ºC, 25 ± 1ºC and 20 ± 1ºC temperature resulted 09, 08 and 04% nymphal loss respectively (Fig. 01). Similarly, the mortality up to adult emergence at aforesaid temperatures ($Y = 6052.x + 18.98R^2 = 0.730$, Fig.02; $Y = 4526.x + 17.15$, $R^2 = 0.648$, Fig.02; and $Y = 4631.x + 10.12$, $R^2 = 0.839$, Fig.02) also yield positive correlation coefficient with increase in concentration. The total nymphal loss up to adult emergence following the topical application of the highest selected sublethal concentration (0.008%) at temperature 30 ± 1ºC temperature was 47%, whereas the total nymphal death up to adult emergence at 25 ± 1ºC and 20 ± 1ºC temperature was 40 and 32% respectively (Fig. 02). These results are like those reported by Eger et al. (1998) in which the mortality of *Franklinella* species showed concentration dependent response. Toews and Subramanyum (2003) also reported the mortality of *Trilobium casteneum* that increased with increasing dosage of insecticide. Similar trend in mortality was also recorded by Pruthi and Jerath (1960) where the toxicity of endrin against *Dysdercus cingulatus* and *Chrotogonous trachypterus* adults at different temperature was accelerated with rise in temperature. Agarwal and Tilak (2006) studied the efficacy of imidacloprid gel bait (2.15%) up to 8 weeks against German cockroach and reported that there was 96.8 to 98.8% reduction in the population as compared to control. Pruthi and Jerath (1960) also determined the effectiveness of endrin against *Dysdercus cingulatus* and *Chrotogonous trachypterus* adults at different temperature and found that the toxicity is accelerated with rise in temperature. Uddin and Ara (2006) studied the toxicity of six insecticide on *Trilobium casteneum* (Herbst) at the temperatures of 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32ºC and observed that there was a marked decrease in the effectiveness of all insecticide tested with a lowering of temperature. The order of toxicity of insecticide was chloropyrifos > lamda-cyhalothrin > Malathion > cypermethrin > carbosulfan > dicofol. Ming and Qiang (2002) studied the selective toxicity of six different insecticides against *Myzus persicae* and recoded the order of toxicity as imidacloprid + SV1 > imidacloprid > endosulfan > methomyl > fanvalerate > dimethoate. Ateyyat et. al. (2009) compared the toxicity of aqueous extracts of nine plants with imidacloprid against sweet potato whitefly, *Bemissia tabaci* and found to killed 71% of early-stage nymphs, which was not significantly different from mortality caused by imidacloprid. Khowaja et. al. (1995) reported that the topical application of 10, 08, and 06 ppm of temik (aldicar) to the 4th instar nymphs of *Dysdercus cingulatus* caused 50, 41, and 27% more nymphal loss than that of control 2% respectively. The initial mortality following the topical application of higher sublethal concentrations (i.e., 80 and 60 ppm) of cythion on 4th and 5th instar nymphs of *D. Cingulatus* was very high within 24 hrs., however, the subsequent mortalities up to adult emergence were insignificant. The total nymphal loss and survival duration of the treated insects were directly proportional and increased linearly with the increase in concentration, whereas the fecundity & fertility of the adults were inversely proportional and decreased linearly with increase in concentrations (Khowaja et al. 2001 and Khowaja and Qamar, 2005).

Many factors such as crowding (Kawada 1989; Matsumura 1996), host plant quality (Lees 1966; Lu and Chen 1993), photoperiod (Joknson 1966; Uddin and Baqui 1993), temperature (Liu and Wu 1994; Joknson 1966), hormones (Zera and Tobe 1990; Fairbairn 1994) and seasonal changes (Muraji et al., 1989) could affect morphogenesis and resulted in wings or leg deformities. In the present investigation, the effect of imidacloprid on malformation was dose as well as temperature dependent. The regression between concentration strength and percentage of malformed adults at 30 ± 1ºC (Y = 3084.2x + 4.705, R^2 = 0.901, Fig.03), at 25 ± 1ºC (Y = 1852.6x + 1.849, R^2 = 0.951, Fig.03) and at 20 ± 1ºC temperature (Y = 2147.4x + 4.150, R^2 = 0.871, Fig.03) yield a positive correlation. Higher percentage (i.e., 23%) of malformed adults was recorded at 20 ± 1ºC temperature, while the malformation at 25 ± 1ºC and 30 ± 1ºC temperature was 13 and 17% respectively (Fig. 03). Tiwari et al. (2006) noted that the topical application of neem-based insecticides on *Dysdercus koenigii* caused ecdysial stasis and development of adultoids and imagoes with varied degree of deformities. Topical application of azadiractin through contact method evoked various specific and non-specific effects during the course of development in various stages of red cotton bugs (Koul, 1984). RD-9 Replin at higher concentration prolonged the nymphal period and affected the emergence of adults of *Dysdercus koenigii* (Gupta et al., 1997). Khowaja et al. (1995) reported that the topical application of temik (aldicarb) to the 4th and 5th instar nymphs of *D. Cingulatus* at 10, 08, and 06 ppm dose produced 7.1, 6.3, 4.8% and 9.6, 8.2, 7.0% adults with malformed wings respectively. Fakhari et al. (2011) studied the comparative toxicity of conventional (imidacloprid) and non-conventional (multineem) insecticides on the biology of *Dysdercus koenigii* and observed that the percentage of malformed adults having folded, twisted wing were very highest (55.38%) in case of imidacloprid treatment as compared to multineem treatment. The topical application of 0.004% monocrotophos on the 4th instar nymphs of *D. koenigii* resulted 30.13% malformation in emerged adults (Fakhari et al., 2011).

The percentage of egg laying, and egg hatched of the female emerged from the 4th instar treated with different sublethal concentrations of imidacloprid were also adversely affected at all tested temperature. The regression between percentage of egg laid and concentrations strength at 30 ± 1ºC (Y= -7412.5x + 87.44, R^2 = 0.868, Fig.04), 25 ± 1ºC (Y = -6690.9x + 90.98, R^2 = 0.925, Fig.04), and 20 ± 1ºC (Y = -4843.7x + 93.35, R^2 = 0.896, Fig.04) showed negative correlation coefficient. The females emerged from the survived 4th instar treated nymphs treated with 0.008% of imidacloprid at 20 ± 1ºC temperature laid lesser number of eggs as compared to at 25 ± 1ºC and 30 ± 1ºC temperature. Following the topical application of 0.008, 0.006, 0.004, 0.002 and 0.001% imidacloprid/4th instar nymphs at 20 ± 1ºC temperature, the egg production of the females emerged from the survived treated nymphs was reduced by 77.9, 71.3, 52.8, 39.4 and 28.2% respectively as compared to control (5.1%), however the topical application of the same concentrations at 25 ± 1ºC and 30 ± 1ºC temperature resulted 69.5, 66.6, 51.7, 39.9, 25.8% and 64.1, 60.1, 52.4, 37.3, 22.8% reduction in egg production of the survived females. Reduced fecundity of *D. koenigii* affected with sublethal concentrations of imidacloprid is consistent with previous studies investigating other hemipterous insects. The fecundities of two aphid species (*Myzus persicae* and *M. nicotiana* Blackman), two green leafhopper species (*Nephotettix virescens*, Distant and *N. cincticeps* Uhler) and a brown planthopper species (*Nilaparvata lugens* Stal) were reduced by more than 50 % after feeding on plants/leaves treated with sublethal concentrations of imidacloprid (Divine et al., 1996; Widiarta et al., 2001; Bao et al., 2009). Similarly, Lashkari et al. (2007) reported that the oviposition rate, longevity and growth of cabbage aphid, *Brevicoryne brassicae* L., were negatively affected by sublethal concentration of imidacloprid. Nishi and Takahashi (2002) observed that the average numbers of egg laid per ovipositing female of *Amphibolous venator* increased with increasing temperature (i.e., 25ºC, 27.5ºC, 30ºC 32.5ºC and 35ºC/ 60-70%RH and 16L-8D of photo regime), however differences were not significant. RH 5849, an analogue of halofenozide, was shown to inhibit ovariole development in Lepidoptera and some Coleoptera (Wing et al., 1988). Monthean and Potter (1992) found that feeding on foliage treated with RH 5849 (1,2-dibenzoyl-1-tertbutylhydrazine) reduce the fecundity of Japanese beetles by 66-74%, but did not reduce the percentage of hatch. Khowaja et al. (1993) reported that the topical application of 2500 ppm, 5000 ppm and 10,000 ppm cythion/larva of *Spodoptera litura* resulted in 10.75, 13.32, and 18.23% reduction in egg production respectively. Aller and Ramsay (1988) also observed that ingestion of RH 5849 by newly emerged adults of several phytophagous beetle species prevented oviposition due to the absence of developing oocytes, whereas, the ingestion of RH 5849 by older female caused cessation of oviposition due to the resorption of the oocytes. Destruction of egg chamber after treatment with tepa, metapa, apholate, hempa, methlmethane sulphonate and hydroxy urea has been reported by Morgan and La Brecque, (1964); Morgan, (1967); Kissam et al., (1967). The effects on division of oogonia in the germarium, the inhibition of growth of oocytes and trophocytes, degeneration of follicular epithelium and the resorption of contents of egg chamber has been reported by Rezabova and Landa, (1967); Landa and Matol, (1971); Khowaja et al. (2001) and Khowaja and Qamar, (2005). The application of 06, 04, and 02 µg of 22, 25-dideoxyecdysone to the 4th instar nymphs of *Dysdercus cingulatus* reduced the egg production of the emerged females by 30.9, 18.5, and 11.0% respectively as compared to control, however, the application of the corresponding doses of 2,22,25 trideoxy-5βhydroxyecdysone decrease the fecundity by 27.6, 21.4, and 15.4% respectively as compared to control (Khowaja et al., 1996). The fecundities of two aphid species (*Myzus persicae* and *M. nicotiana* Blackman), two green leafhopper species (*Nephotettix virescens*, Distant and *N. cincticeps* Uhler) and a brown planthopper species (*Nilaparvata lugens* Stal) were reduced by more than 50 % after feeding on plants/leaves treated with sublethal concentrations of imidacloprid (Divine et al., 1996; Widiarta et al. 2001; Bao et al., 2009). Similarly, Lashkari et al. (2007) reported that the oviposition rate, longevity and growth of cabbage aphid, *Brevicoryne brassicae* L., were negatively affected by sublethal concentration of imidacloprid. Boina et al. (2009) also observed that the fecundity and fertility of the Asian citrus psyllid, *Diaphorina citri,* were reduced significantly following the long-term exposure of imidacloprid through systemic method. Nishi and Takahashi (2002) observed that the average numbers of egg laid per ovipositing female of *Amphibolous venator* increased with increasing temperature (i.e., 25ºC, 27.5ºC, 30ºC 32.5ºC and 35ºC with constant 60-70%RH and 16L-8D of photo regime), however the differences were not significant.

Similarly, the percentage of egg hatching of the effected female emerged from the survived treated 4th instar nymphs also reduced linearly ($Y = -8779.4x + 85.34$, $R^2 = 0.884$, Fig.05; $Y = -10915.0x + 77.67$, $R^2 = 0.818$, Fig.05; $Y = -7618.9x + 44.12$, $R^2 = 0.336$, Fig.05 at 30 ± 1ºC, 25 ± 1ºC and 20 ± 1ºC temperature respectively) yielded a negative correlation coefficient. The hatching of the eggs laid by the affected females at 20 ± 1ºC temperature emerged following the topical application of 0.008, 0.006, 0.004, 0.002 and 0.001% imidacloprid on 4th instar nymphs dropped by 65.8, 50.2, 51.9, 42.9, and 36.9% respectively, however the hatching of the eggs at 25 ± 1ºC and 30 ± 1ºC temperature following the application of same concentrations was reduced by 38.4, 35.5, 28.0, 17.4, 17.8% and 39.6, 30.1, 21.3, 15.3, 13.1% respectively as compared to control (Fig. 05). Boina et al. (2009) also observed that the fecundity and fertility of the Asian citrus psyllid, *Diaphorina citri,* were reduced significantly following the long term exposure of imidacloprid through systemic method. The topical application of various sublethal concentrations of monocrotophos to 4th and 5th instar nymphs of *D. cingulatus* resulted in linear fall in egg production and egg hatching and showed a negative correlation with the increasing concentrations (Khowaja et al., 1994; Khowaja and Qamar, 2002). Likewise, the average fertility of the eggs laid by the affected females of *D. Cingulatus*, after the topical application of higher selected doses (06, 04, 02 µg of 22, 25-dideoxyecdysone) on 4th instar nymphs, decreased by 35.3, 30.1, and 26.0% respectively as compared to control, however, with corresponding dose of 2,22,25trideoxy-5βhydroxyecdysone, the hatchability of the eggs was dropped by 29.2, 20.3, and 13.8% respectively as compared to control (Khowaja et al., 1996). Inhibition in fecundity and fertility after the application of natural ecdysones or their analogues have also been reported by Jalaja et al. (1976), Ahmad & Khan (1985), Ahmad (1983), and Khan et al. (1990). Wright et. al. (1971) observed that β-ecdysone prevented lipid synthesis which is necessary for vitellogenesis. On the contrary, Zeng and Wang (2010) reported that the exposure of sublethal dose of imidacloprid to *Tetranychus cinnabarinus* led to a significant increase in the hatch rate of eggs. James and Price (2002) also reported that the two spotted spider mites, *Tetranychus urticae,* either directly exposed to spray formulations or fed on discs cut from a systemically treated bean plant produced 10-26% more eggs during the 1st 12day of adult life and 19-23% more during adult hood compared with a water only treatment.

ANALYTICAL FIGURES:

Fig. 01: Fitted linear regression line showing initial mortality following topical application of sublethal concentrations of Imidacloprid at three different temperatures.

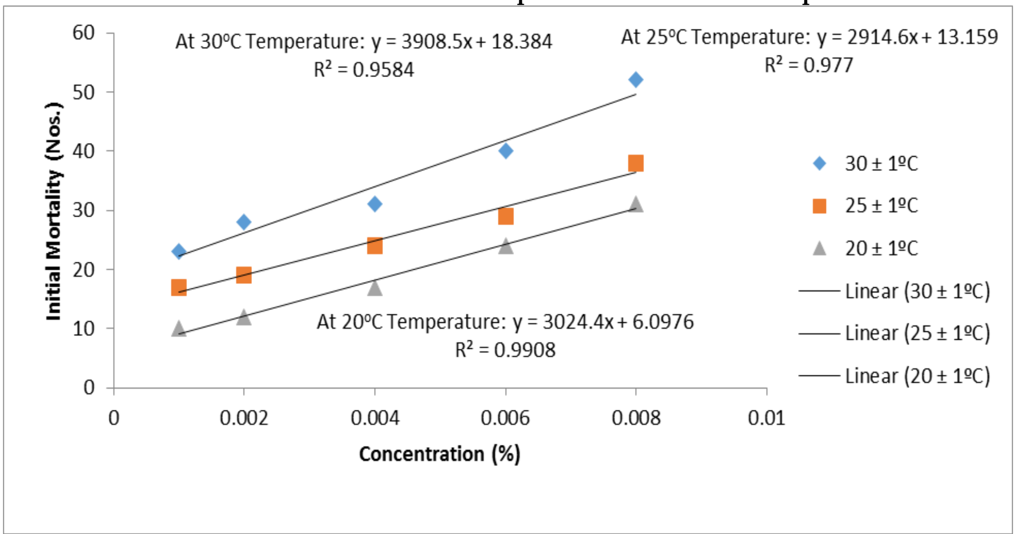


Fig. 02: Fitted linear regression line showing total mortality up to adult emergence following topical application of sublethal concentrations of Imidacloprid at three different temperatures.

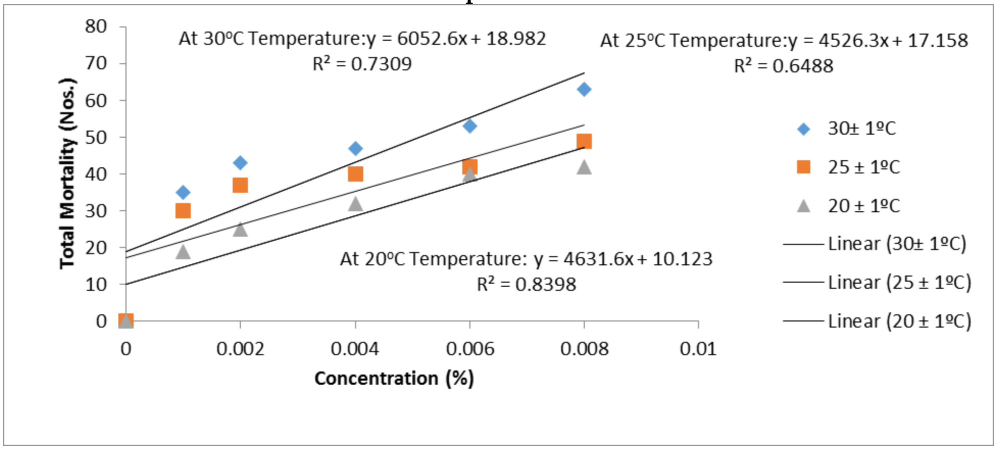


Fig. 03: Fitted linear regression line showing malformation following topical application of sublethal concentrations of Imidacloprid at three different temperatures.

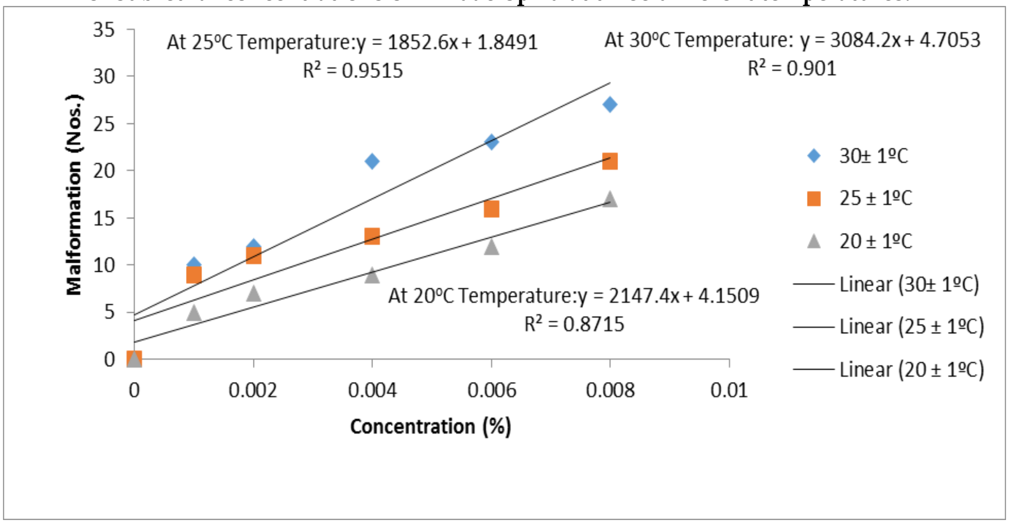


Fig. 04: Fitted linear regression line showing percentage of egg laid following topical application of sublethal concentrations of Imidacloprid at three different temperatures.

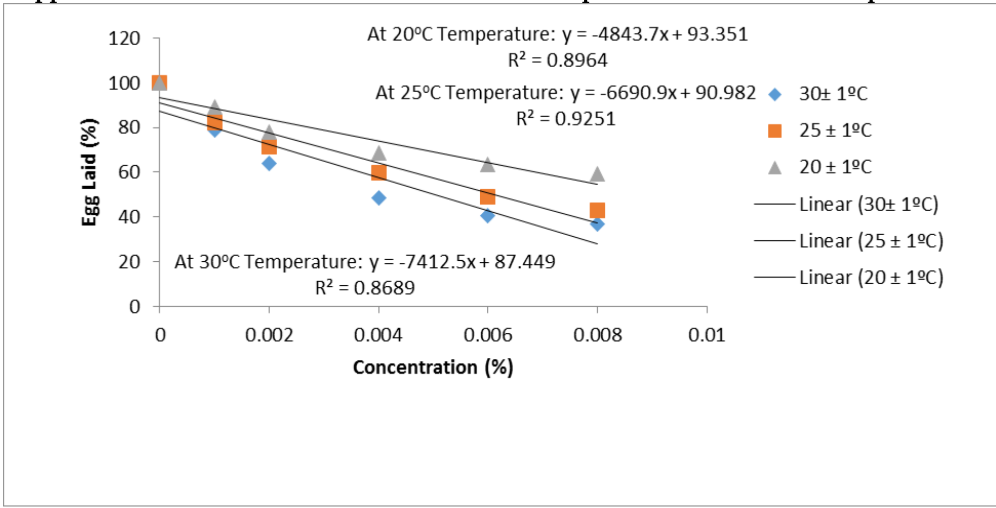
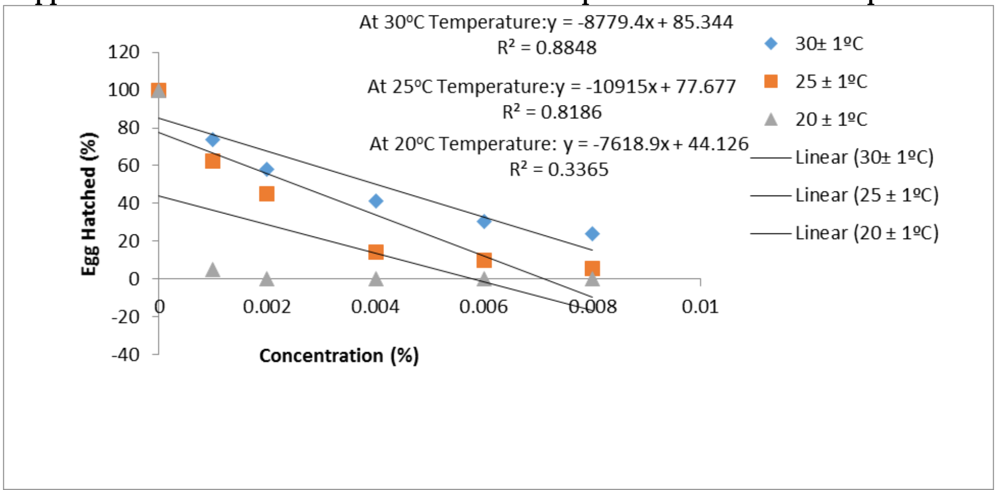


Fig. 05: Fitted linear regression line showing percentage of egg hatched following topical application of sublethal concentrations of Imidacloprid at three different temperatures.



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