

Influence of post treatment temperature on the toxicity of four macrolacton insecticides against *Spodoptera littoralis* (BoisduVal) (Lepidoptera:Noctuidae)

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Abstract

Temperature is a main ecological factor affect the bioinsecticides toxicity on the destructive larval stage of the cotton leafworm (CLW). The effect of post-exposure temperatures from 13 to 39 °C on the toxicities of two spinosyns (spinosad and spinetoram) and two avermectins (abamectin and emamectin benzoate) toward *Spodoptera littoralis* (BoisduVal) larvae was evaluated using topical and feeding bioassays. Spinosad and spinetoram showed negative temperature coefficient against CLW larvae. The LD₅₀ values of spinosad and spinetoram increased by 70.21 and > 81.63 folds when temperature increased from 13 to 39 °C. These two compounds also showed negative temperature coefficient values (-1.71; - 9.92) in the feeding bioassay. On contrast, in feeding application abamectin and emamectin benzoate showed high positive temperature coefficient (27.79 and 194.50) when temperature increased from 19 to 39°C. The present results ascertain the effect of temperature on the pesticides toxicity. So, spinosyns should be applied in cold weather, whereas, abamectin and emamectin benzoate performed well in relative high temperature.

Keywords: toxicity, spinosad, spinetoram, abamectin, emamectin benzoate, temperature coefficient, *Spodoptera littoralis*.

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Introduction

Temperature is considered a biotic factor affecting biological processes in all living organisms especially on poikilothermic organisms like insects (Fand et al., 2015; Amarasekare & Edelson, 2004; Bale et al., 2002). Also, it is a major factor affecting on insecticide toxicity (DeVrise & Georghiou, 1979). Also, toxicant exposure time, humidity and insecticide formulation affect insecticide toxicity. The relationship between temperature and insecticide efficacy has been varied depending on insecticide molecule, insecticide mode of action, target species, method of application and amount of insecticide ingested or contacted (Kavallieratos et al., 2009; Scott, 1995; Johnson, 1990). The correlation coefficient between insecticide and temperature can be either positive or negative. This coefficient was extensively studied in different species (Khan & Akram, 2014; Glunt et al., 2013; Vassilakos & Athanassiou, 2013; Kowalska, 2008; Mannaa & El-Ghareeb, 1992). The variation between the highest and lowest temperature degree in Egypt through the year is too much. The range of temperature degrees in Egypt is very varied significantly from summer to winter and from day to night. In addition to the economic importance of CLW, *S. littoralis* as one of the most serious injuries insect pests on fields crops as well as horticultural crops in greenhouse or open field not only in Egypt but also on the most Mediterranean countries (Abdu-Allah et al., 2009; El-Gahreeb et al., 2009; Abdu-Allah, 2007). This insect has not any diapause or/and hibernation or aestivation in Egypt. Consequence, CLW can be adapted in different regions in Egypt and can tolerate the severity temperature. It is recorded from 3 to 46 °C in night winter and day summer. The use of insecticides is still the major and

familiar method for controlling the CLW. The deposit of insecticide faces the same insect weather in the field. In last decades the new groups of insecticides; like macrolacton group has introduced and recommended for controlling CLW larvae in Egypt (Anonymous, 2016). Macrolactone insecticides are known as microbial bioinsecticides, derived from actinomycetes bacterium species (Copping & Menn, 2000; Putter et al., 1981). Spinetoram and emamectin benzoate are the second generation of spinosad and abamectin. Spinosad is a microbial origin, macrocyclic lacton glycoside, derived from actinomycete bacterium species, *Saccharopolyspora spinosa* Mertz and Ya (Sparks et al., 1998). Emamectin benzoate is a modified fermentation product of the soil microorganism, *Streptomyces avermitilis*. Spinosyn and avermectin insecticides affect the insect nervous system. Abamectin increases the flux of chloride ion at the neuromuscular junction in the nervous system, due to this causes, the arthropods start cessation of feeding and irreversible paralysis (Ishaaya et al., 2002). Spinosad changes the function of GABA-gated chloride channels and nicotinic acetylcholine receptors (Salgado et al., 1997). Although biopesticides have high efficiency and friendly using, they are highly sensitive to the environment (Scott, 1995). In applicable where comparable products from many insecticide classes are ready, temperature should be investigated as a factor in the decision-making steps. Determination of a product temperature coefficient will enable pest managers to select a compatible product that is efficacious under the given environmental conditions. The four tested insecticides are available on the Egyptian market and most of these are recommended for controlling CLW in Egypt (Anonymous, 2016; Abdu-Allah,

2015). The objective of this study to investigate the effect of four post-temperature degrees (13, 19, 29, 39 °C) on the toxicity of the four macrolacton using topical and feeding bioassays against the fourth instar larvae of the *S. littoralis* under constant conditions.

Materials and methods

Insects: Strain of CLW, *S. littoralis* used in this study was maintained in Plant Protection Laboratory, Assiut University, Egypt for more than fifteen years without exposure to insecticides. It is reared on castor leaves as described by (Eldefrawi et al., 1964).

Insecticides: Spinosad (Spintor[®], SC 24 %, Dow AgroSciences Co.); spinetoram (Radient[®], SC12 %, Dow AgroSciences Co); abamectin (Gold[®], EC 1.8 %, ELHELB Pesticides & Chemicals Co., Egypt); and emamectin benzoate (Radical[®], EC 0.5 %, Agromen Chemicals Co., Ltd.) were used. These insecticides are provided from Experimental Agricultural Research Faculty of Agriculture, Assiut University, Assiut, Egypt except Spintor[®] which was bought from commercial insecticide market in Egypt.

Bioassay techniques: The topical and feeding bioassays were done at Plant Protection Department Laboratories, Faculty of Agriculture, Assiut University, Egypt according to (Abdu-Allah, 2011). For topical application, spintor[®] and radient[®] were dissolved in glacial acetic acid and acetone mixtures (1:1), while acetone solvent was used for gold[®] and radical[®]. For feeding bioassay, triton x-

100 (0.05%), as detergent, and distilled water as solvent were used. Parts of castor bean leaves were dipped in the pesticide solution for 5 seconds, left until dry. To each replicate, ten selected larvae were fed for 24 h on the treated castor bean leaves, and then the larvae were allowed to feed for 24 h on untreated castor bean leaves. For both bioassays, five to six serial concentrations of each compound was tested against the 4th instar larvae of CLW (the average weight of larvae was 32–36 mg). Separately, to every temperature tested (13, 19, 29, 39 °C), the treatments were incubated in adjusted gross chamber in dark and 55± 5% RH for 72 h till recording mortality results. The experiment of each tested compound was duplicable repeated. Percentages of mortality were corrected by Abbot's formula (Abbott, 1925), then pooled and analyzed by probit analysis using the software SPSS (Version 10.0 for windows, SPSS Inc., Chicago, the USA) to determine median lethal concentrations (LC₅₀/ LD₅₀), slope and χ^2 values. According to (Litchfield & Wilcoxon, 1949) the toxicity of bio-pesticides was considered significantly different if the confidence intervals (CIs) at LD₅₀/LC₅₀ level did not overlap. Temperature coefficients of each insecticide were recorded at different temperatures according to (Musser & Shelton, 2005).

Results

Data of topical application showed that, both spinosyn insecticides had significantly negative temperature coefficients on for *S. littoralis* at all temperature tested. The LD_{50S} value of

spinosad gradually increased with the increase of tested temperatures. These values were 8.94, 118.64, 314.94 and 627.62 $\mu\text{g a.i. Larvae}^{-1}$ at 13, 19, 29 and 39 °C, respectively. This means, the spinosad LD₅₀ value that determined at 13°C was less than that recorded at 19, 29, 39 °C by 13.27, 35.23, 70.21 times, respectively. Spinosad showed significant negative temperature coefficient and its toxicity was significantly high at low temperature, and decreased by the increase in temperature degrees. As for spinetoram, the LD_{50s}

values took the same trend; these recorded 0.49, 3.04, and 18.35 $\mu\text{g a.i. Larvae}^{-1}$ at 13, 19, and 29 °C, respectively. However, at 39 °C the tested doses of spinetoram had not any larval mortality. In another words spinetoram have significantly negative temperature coefficient with -37.45, -6.20. Also as in spinosad, spinetoram showed high toxicity at 13 °C and the efficiency declined by the increase in temperature, the compound showed no toxicity at 39 °C tested (Table 1 & Fig. 1).

Table 1: LD₅₀, slope values of LDP lines of certain bio-insecticides toxicity for *S. littoralis* larvae using topical bioassay.

Bio-insecticide	Temp (°C)	n ^a	LD ₅₀ (95% CL) ($\mu\text{g a.i.larva}^{-1}$)	Slope (\pm SE)	χ^2 , (df)	Temp coefficient ^b				
						6 °C	10 °C	16 °C	20 °C	26 °C
Spinosad	13	360	8.94 (0.32-58.93)	0.28 (\pm 0.24)	4.42 (5)	-13.27				
	19	360	118.64 (67.10-138.76)	4.69 (\pm 1.21)	0.64 (5)		-2.65			
	29	360	314.94 (77.83-748.40)	2.67 (\pm 0.78)	0.03 (2)			-35.23		
	39	360	627.62 (NE)	2.06 (\pm 0.81)	0.01 (2)				-5.29	-70.21
Spinetoram	13	238	0.49 (0.32-5.89)	0.40 (\pm 0.19)	4.54 (3)	-6.20				
	19	240	3.04 (NE)	1.12 (\pm 0.19)	6.34 (5) ^c					
	29	240	18.35 (8.69-50.80)	1.91 (\pm 0.33)	0.70(2)		-6.04			-37.45
	39	240	>40 (NE)	(NE)	(NE)					
Abamectin	13	210	1.86 (1.56-2.21)	1.23 (\pm 0.15)	0.45(4)	-1.81				
	19	210	3.37 (1.97-7.25)	1.11 (\pm 0.17)	4.08(4)		+1.36			
	29	210	2.47 (1.47-4.17)	0.96 (\pm 0.16)	7.15 (4) ^c			-1.33		
	39	210	1.93 (1.13-3.39)	1.00 (\pm 0.19)	11.38(4) ^c				+1.75	-1.04
Emamectin benzoate	13	210	0.05 (0.03-0.07)	2.98 (\pm 0.51)	1.94 (5)	+1.25				
	19	210	0.04 (0.02-0.07)	2.22 (\pm 0.68)	0.13 (2)		+2.00			
	29	210	0.02 (0.002-0.04)	3.15 (\pm 0.71)	2.09 (2)			+2.50		
	39	210	0.01 (0.002-0.03)	1.87 (\pm 0.69)	0.98 (2)		+2.00		+4.00	+5.00

^a Total number of *S. littoralis* tested for each temperature/insecticide treatment. ^b Ratio of higher to lower LD₅₀ value for 6–10, 16–20 and 26°C differences in temperature. A negative coefficient indicates a higher LD₅₀ at the higher temperature.

^c χ^2 significantly different from expected ($P < 0.10$), (NE): Confidence limit of LD₅₀ not estimated.

In contrast the above mentioned results, abamectin and emamectin benzoate showed significant positive temperature coefficients when used topically on *S. littoralis* larvae. The temperature coefficient values of emamectin benzoate were gradually increased with increasing temperature. Emamectin benzoate has significant temperature coefficient of +5 and +2.5, required an emamectin dose 5, and 2.5 times lower at 39, and 29, °C, respectively than needed at 13 °C to get

the same control result. From 19- 39 °C, the LD₅₀ values of abamectin decreased gradually; with 3.37, 2.47, and 1.93 $\mu\text{g a.i. larvae}^{-1}$ at 19, 29 and 39 °C, respectively. Abamectin has lower positive temperature coefficient than emamectin benzoate with +1.36 and +1.75 at 10, and 16 °C temperature (Table 1 & Fig.1). The feeding application results data presented in table 2 showed that two spinosyns have negative temperature coefficient in all tested

temperature except for 39 °C. The LC₅₀ values of spinosad and spinetoram were increased gradually from 13 to 29 °C, with 324.72,; 504.36, 553.92 and 125.16, 461.31, 1242.00 mg a.i. liter⁻¹ at 13, 19, and 29°C, respectively. The temperature coefficients of spinosad, and spinetoram

were -1.71, - 9.92; and -1.55, - 3.67 at 29, 19 °C, respectively. This means to obtain the same mortality at 13 °C spinosad and spinetoram concentration should be increased by 1.71, 9.92; and 1.55, 3.67 folds than that at 29, 19 °C, respectively.

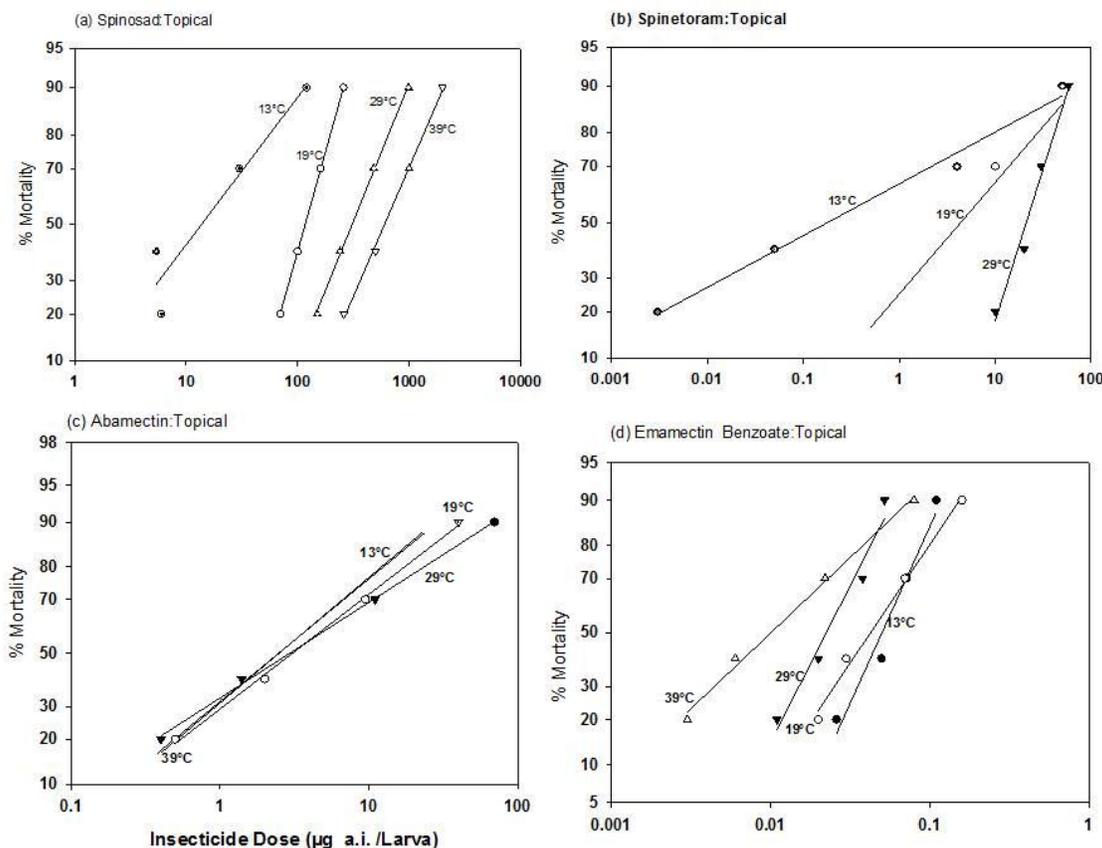


Figure 1: Relationship between log insecticide doses (ug a.i. Larva-1) of spinosad, spinetoram, abamectin, emamectin benzoate and mortality of 4th instar larvae of *S. littoralis*, using topically application method on constant tested temperatures post treatment.

For two avemectin tested the LC₅₀ values of abamectin and emamectin benzoate decreased gradually from 13 to 39 °C, with 2569, 715.8, 395.02, 185.05, and 3.89; 0.27; 0.14; 0.02 mg a.i. liter⁻¹ at 13, 19, 29, and 39°C, respectively. Emamectin benzoate showed higher significant positive temperature coefficient than that recorded for

abamectin. Emamectin benzoate and abamectin with temperature coefficient of +194.50, +13.88; +13.5,+3.87 and +27.79, +6.50, required an emamectin and abamectin concentrations 194.5, 13.88; 13.5, 3.87; and 27.79, 6.50 times lower at 39, 29,19 °C, respectively that required at 13 °C to get the same larval control level (Table 2 & Fig. 2).

Table 2: LC₅₀, slope values of LCP lines of certain certain bio-insecticides toxicity for *S. littoralis* larvae using feeding bioassay.

Bio-insecticide	Temp		LC ₅₀ (95% CL) (mg a.i. liter ⁻¹)	Slope		Temp coefficient ^b				
	(°C)	n ^a		(±SE)	γ ₂ , (df)	6 °C	10 °C	16 °C	20 °C	26 °C
Spinosad	13	300	324.72 (205.42-503.32)	6.87 (±1.90)	2.33 (2)	-1.55				
	19	300	504.36 (264.39-2553.69)	2.98 (±1.33)	0.93 (2)			-1.09		
	29	300	553.92 (272.90-8560.68)	4.02 (±1.67)	1.32 (2)				-1.71	
	39	300	282.89 (201.53-511.81)	5.32 (±1.47)	0.11 (2)			+1.95		+1.78 +1.15
Spinetoram	13	300	125.16 (86.25-172.33)	3.78 (±1.77)	3.52 (2)	-3.67				
	19	300	461.31 (337.59-790.69)	5.89 (±0.77)	0.49 (2)			-2.69		
	29	300	1242.00 (571.09-3387.42)	4.46 (±1.53)	0.22 (2)				-9.92	
	39	300	39.68 (15.13-50.44)	7.01 (±2.74)	0.04 (2)			+31.30	+11.63	+3.15
Abamectin	13	300	2569.00 (NE)	4.23 (±3.07)	0.01 (2)	+3.59				
	19	300	715.80 (404.65-6718.69)	4.96 (±1.82)	0.02 (2)			+1.81		
	29	300	395.02 (156.33-1416.43)	3.95 (±1.65)	0.15 (2)				+6.50	
	39	300	185.05 (8.82-345.09)	3.55 (±1.68)	0.21 (2)			+2.13		+3.87 +13.88
Emamectin benzoate	13	300	3.89 (2.39-4.74)	1.13 (±0.20)	1.73 (2)	+14.41				
	19	300	0.27 (0.05-0.51)	0.82 (±0.16)	2.69 (2)			+1.93		
	29	300	0.14 (0.02-0.29)	0.93 (±0.16)	1.73 (2)				+27.79	
	39	300	0.02 (0.001-0.03)	1.87 (±0.69)	0.98 (2)			+14.00		+13.50 +194.50

^aTotal number of *S. littoralis* tested for each temperature/insecticide treatment. ^bRatio of higher to lower LC₅₀ value for 6–10 ,16-20 and 26°C differences in temperature. A negative coefficient indicates a higher LC₅₀ at the higher temperature, (NE): Confidence limit of LC₅₀ not estimated.

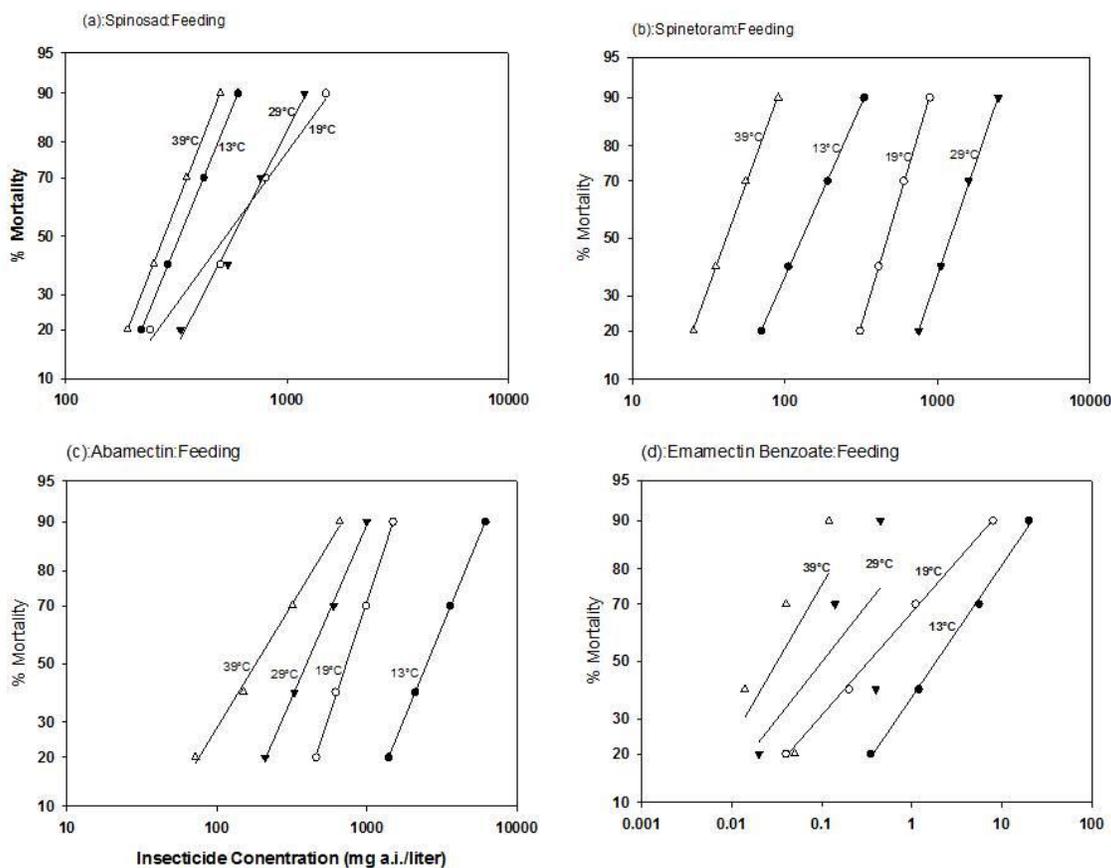


Figure 2: Relationship between log insecticide concentrations (mg a.i.liter⁻¹) of spinosad, spinetoram, abamectin, emamectin benzoate and mortality of 4th instar larvae of *S. littoralis*, using feeding bioassay method on constant tested temperatures post treatment.

The slope values did not significantly differ among tested temperatures for any of the insecticides except for spinosad when tested topically at 13°C, so that temperature coefficients similar to those reported for LC₅₀ could be calculated for all lethal concentrations. The slope values are as steep as frequently encountered in insecticide assays. This was likely due to an observed no repellent effect of the insecticides at the higher doses. In topical application, it was found high significant positive

correlation (+0.99) between temperatures tested and the LD₅₀ values of spinosad and spinetoram, however there were high negative correlation with emamectin benzoate and no significant correlation with abamectin. Converse results were found in feeding bioassay, the significant negative correlation was detected with abamectin and emamectin benzoate (-0.83, 0.74), while spinosad and spinetoram showed no significant correlations (-0.16, +0.07) (Table 3).

Table 3: Correlationship between temperature degrees (13, 19, 29, 39 °C) and LD₅₀ or LC₅₀ values of spinosad, spinetoram, abamectin and emamectin benzoate tested on the 4th instar larvae of *S. littoralis*.

	Temperature	
	LD ₅₀	LC ₅₀
Spinosad	+0.99**	-0.16 ^{ns}
Spinetoram	+0.99**	+0.07 ^{ns}
Abamectin	+0.11 ^{ns}	-0.83*
Emamectin benzoate	-0.99**	-0.74*

** High significant, * significant, ^{ns} no significant at p=0.05.

Discussion

In the present study the influence of four constant temperatures levels on the effectiveness of different insecticides in CLW larvae was determined under laboratory conditions. Management of this serious injuries larval insect is considered on the field and horticultural crops in Egypt as well as in the most Mediterranean countries (Aydin & Gürkan, 2006; Musser & Shelton, 2005; Kandil et al., 2003). Most recommended insecticides used against CLW larvae usually caused mortality by confusing functions of the nervous system (Khan et al., 2003). Since an insect's body temperature changes with its surroundings, environmental temperature can affect the toxicity of the insecticide (Glunt et al., 2013). Variable metabolic

enzymes in insects' body are responsible for the detoxification of insecticides. Natural functioning of the nervous system, is greatly temperature dependent (Montgomery & Macdonald, 1990). In the present results, the spinosyn insecticides exhibited a negative association with surrounding temperatures. Therefore, these could be assumed theoretically to be more toxic in low temperature conditions. The results are in convinced with those reported on spinosyn insecticides in different insect pests (Khan & Akram, 2014; Musser & Shelton, 2005). Conversely to the present results, other studies reported that temperature factor has positively effected on spinosad toxicities such as spinosad against grasshoppers (Amarasekare & Edelson, 2004), *Callosobruchus maculatus* (Sadat & Asghar, 2006). This

may be due to the variation in the morphological and physiological characters of insect species. The high potency of spinosad and spinetoram at low temperature can be explained by two suggestions. The first, at low temperature the molecules of these insecticides decreased through the body cuticle so that protection insecticide molecules for intensive exposition to a biological process called biotransformation. Multiple enzymatic activities are responsible for different chemical forms in any xenochemical compound such as spinosad and spinetoram through biotransformation (Harwood et al., 2009). Second suggestion by (Weinzierl et al., 1998) who stated that temperature is an important factor in affecting the effectiveness of microbial insecticides, since spinosad and spinetoram are microbial insecticides which might be a possible factor for decreasing the toxicity at higher temperatures. Being of the view of (Khan & Akram, 2014) further research should be done to understand the phenomenon of decreased potency of spinosad and spinetoram with the decrease in temperature levels. Theoretically, keeping in view the negative relationship between temperature and toxicities of the spinosyns, these insecticides should provide better at low temperatures in the field. Therefore, spinosyns insecticides should be applied in colder winter climates for controlling *S. littoralis* larvae. The tested avermectin insecticides showed positive temperature coefficient against cotton leafworm larvae in both bioassays. The results reported in this study agree with those of (Boina et al., 2009) who reported that abamectin toxicity increased with the increase in

temperature from 17 to 37 °C in psyllid, *Diaphorina citri* with the Petri dish bioassay. Emamectin benzoate results in present investigation relatively compatible with research reported by (Khan & Akram, 2014) who found that emamectin benzoate has a positive temperature effect against *Musca domestica* L. Penetration and metabolic enzyme activities are partially responsible for detoxification of insecticides. The high penetration of abamectin and emamectin benzoate may be responsible for the high toxicity of these compounds as compared with spinosad and spinetoram. Based on the suggestion of (Harwood et al., 2009) who reported that sodium influx increases due to the stability of open sodium channels at low temperatures, it may be suggest that the activity chloride ion flux at glutamate-gated chloride channel as site of action of abamectin and emamectin benzoate (Ishaaya et al., 2002; Dunbar et al., 1998) is significantly influence by sodium influx. The effect of tested insecticides depends on mode of application and temperatures; this statement is cleared by our presentation, where the temperature coefficients are varied in the same tested temperature at different bioassays. It is generally known that at high temperature, higher amount of the insecticide penetrates the cuticle because the increased chemical solubility or reactivity; however the small amount of the toxicant penetrating the cuticle at low temperature is more active due to decreasing metabolism that gives comparatively high effect at low temperature. This generalization has been realized during the present study because the highest toxicity was recorded

at 13°C with spinosad and spinetoram but it was the lowest for avermectins. On the other hand, an increase in temperature means also an increase in the activity of CLW larvae consequence the quick touch of the insecticide at the site of action that tends to perform a positive temperature coefficient of mortality. These results insist that there are negative correlation between temperature degrees and toxicity of spinosad and spinetoram, while positive correlation with abamectin and emamectin benzoate. Spinosad and spinetoram showed negative temperature coefficient, so it should be applied at low temperature, in the winter season for controlling CLW larvae. Otherwise for abamectin and emamectin benzoate this showed positive relation with temperature. So to get better control of CLW larva in field by emamectin benzoate and abamectin, it prefers to apply these compounds in the summer season to give a good control result. Knowledge of a product's temperature coefficient will help pest managers to select a product that is effective under the given environmental conditions (Musser & Shelton, 2005). This investigation needs further studies about the effect of changing temperature in the toxicity of tested insecticides.

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