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Effect of Entomopathogenic Nematodes and Some Pharmaceutical Inhibitors of Eicosanoid Biosynthesis on the Desert Locust *Schistocerca gregaria* (Forskal)

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ABSTRACT

The entomopathogenic nematodes, *Heterorhabditis* and *Steinernema* together with their associated bacteria *Photorhabdus* and *Xenorhabdus*, respectively have biological control potentials. To address some of the fundamental factors underlying the immunocompetence of the host insect *Schistocerca gregaria* (Forskal) following nematode infection, we tested a hypothesis that the insect immune-mediating eicosanoid pathway may be affected by the virulent action of the Egyptian nematode isolate *H. indica* (RM1). Haemocoelic injection of the nematode into the fifth instar nymphs of *S. gregaria* evoked the haemocyte microaggregation and nodulation reactions and increased the mortality percentages of these economically important pests. Separate treatments with specific inhibitors of the phospholipase A2; the cyclooxygenase and the dual cyclooxygenase / lipoxygenase pathways, reduced both haemocyte microaggregation and nodulation reactions, supporting the point of view that nodule formation is a complex process involving both cyclooxygenase and lipoxygenase products. The inhibitory effects of the phospholipase A2 inhibitor, dexamethasone, on microaggregation and nodulation were obviously apparent during the first hour of injection and these effects increased greatly over the following 24h. The dexamethasone effects were expressed in a dose-dependent manner and they were reversed by the co-injection of the nematode-injected insects with the exogenous eicosanoid-precursor polyunsaturated fatty acid, arachidonic acid (C20:4n-6). These findings strongly support the identification of microaggregation and nodulation as specific insect cellular defense reactions that are mediated by eicosanoids. The *S. gregaria* nymphs contain trace levels of the eicosanoid-precursor polyunsaturated fatty acids in six different tissues as detected by mass spectrometry.

Key Words: Entomopathogenic nematodes, Microaggregation, Nodulation, Inhibitors, Eicosanoid, *Schistocerca gregaria*.

INTRODUCTION

The desert locust *Schistocerca gregaria* (Forskal) is a polyphagous sporadically very serious pest allover the world especially in Africa and India. During overcast conditions, hoppers spend nearly all the time on vegetation. The fifth instar nymphs are the most voracious individuals (FAO, 2001).

The entomopathogenic nematodes (EPN) of the families Heterorhabditidae and Steinernematidae with their symbiotic bacteria *Photorhabdus (P.) and Xenorhabdus* (X.), respectively have been used commercially as biocontrol agents of some insect pests, with a clear aim at the avoidance of environmental pollution and health hazards of chemical pesticides. After the entrance of the infective juveniles (IJs) into the selected host insect, they release their symbiotic bacteria into the insect haemocoel where they grow causing septicemia leading to host death (Park *et al.*, 2004). The susceptibility of some lepidopterous insect pests towards infection with nematodes of *Heterorhabditis* species varies greatly according to the nematode species and isolates (Shairra, 2000). Also, the susceptibility of the desert locust to EPN has been studied by very few outhers (Goldsworthy *et al.*, 2003 and Khalied Ibn El-Waleed, 2006).

Insects are able to defend themselves against foreign organisms by their innate immune response. This response is elicited by sequential reactions such as recognition of no self, which leads to cellular and/or humoral immune reactions (Gillespie *et al.*, 1997 and Sicard *et al.*, 2004). The cellular immune response represents a rapid therapeutic method that includes nodule formation, encapsulation and phagocytosis (Brugirard-Ricaud *et al.*, 2005). This response is then reinforced by the humoral immune response that inactivates or kills foreign organisms through the activities of polyphenoloxidase (Dorrah & Ayaad, 2004), of lysozyme (Dunn, 1986), or of attacins, cecropins, and other antibacterial proteins (Ji & Kim, 2004).

Specific chemical compounds present in different organisms can be recognized by insects as no self and is conveyed by localized mediators such as eicosanoids (Stanley-Samuelson, 1994) and biogenic amines (Dunphy & Downer, 1994). Such mediators induce immunocompetent haemocytic cells that exhibit actual immune responses.

Aim of the present work is to elucidate the susceptibility of the locust *S. gregaria* towards invasion with

the nematode isolates *Heterorhabditis indica* (RM_1) (*H. indica*). In addition, to disrupt the immune reactions of *S. gregaria* in a trail to control it by increasing its vulnerability towards the entomopathogenic nematodes.

MATERIALS AND METHODS

Insects

1. *Schistocerca gregaria* (Forskal)

The stock culture was maintained at 30±2°C, 65±5% RH and 16:8 (L:D) h. Detailed descriptions of the rearing techniques are given by Ibrahim (1980).

2. *Galleria mellonella* (L.)

The greater wax moth, *Galleria mellonella* was reared according to the method described by Sheble (2002).

Entomopathogenic Nematodes

The Egyptian nematode strain *H. indica* (RM_1) was isolated from Egypt and was identified by El-Assal *et al.* (2002) and mass culturing described by Bedding & Akhurst (1975) and modified by Shairra (2000) using the last instar larvae of the *G. mellonella* was adapted.

Susceptibility of *S. gregaria* nymphs to the injection with the nematode, *H. indica* (RM_1)

To evaluate the mortality percentages of the nymphs of *S. gregaria* to the injection with different doses of the nematode *H. indica* (RM1), fifth-instar individuals in five groups were cooled at 4°C for 1h prior to surface swabbing with 95% ethanol. Nymphs were then injected with *H. indica* (RM_1) suspensions (20, 15, 10, 5 or 3 IJs / 2 µl distilled water). Injection was carried out using a 30 gauge sterile, siliconized needle fitted to a 50µl syringe (Hamilton, Reno, NV) calibrated to deliver a volume of 2µl. This injection method allows standardizing the level of infection (*i.e.*, the exact number of IJs/nymph). The needle was carefully inserted through the thoracicoabdominal junction under the coxa of the hind leg. This method reduced insect bleeding (Ouedraogo *et al.*, 2002). Four replicates of each treatment were carried out. The number of cadavers was recorded 6, 12, 24, 36 & 48 hours post- injection and the percentage of mortality was calculated to estimate the LD_{50} & LD_{25} values. Negative and positive controls were included with each experimental group to determine the background level. To assess the response in unchallenged individuals, negative controls (intact) were included. The positive control individuals were injected with 2µl distilled water. The experimental treatments were incubated at 30± 2°C (Phelps *et al.*, 2003).

Statistical analysis

Data presented in percentage values in the present study were normalized using arcsine transformation values of LD_{25} and LD_{50} using a software package "Ldp-line" a copyright by Ehab, M. Bakr, Plant Protection Research Institute, ARC, Giza, Egypt. The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($P \leq 0.05$). All analyses were made using a software package "Costat", a product of Cohort Software Inc., Berkeley, California.

Assays for micro-aggregation

Haemocyte micro-aggregation in the nematode-injected nymphs were assessed at a standard time (12h post-injection) except in the time-course experiments. Nymphs were anesthetized by chilling on ice and haemolymph was collected by pericardial puncture using Teflon-lined needles (Gunnarsson & Lackie, 1985). Ten µl of haemolymph were collected, mixed with 80µl of diluting solution (NaCl, 4.65g ; KCl, 0.15g; $CaCl_2$,0.11g ; crystal violet, 0.05g and acetic acid, 1.25ml / liter distilled water). 20µl of diluted haemolymph were applied to a haemocytometer (AO instrument Co., Buffalo, NY). The haemocytes were allowed to settle for 3 min, a coverslip was then applied and the number of cellular microaggregates in each sample was determined by counting four large fields in the haemocytometer using phase-contrast optics. Numbers of micro-aggregates were normalized to micro-aggregates/ml haemolymph. Only haemocyte clusters containing ten cells (about 100µm) or more were considered micro-aggregation *in vivo*. (Jurenka *et al.*, 1997).

Assays for nodulation

Nodulation in the nematode-injected nymphs were assessed at 12h post-injection except in the time course experiments. *S. gregaria* 5th instar nymphs were anesthetized by chilling on ice for 10 min and surface sterilized with 95% ethanol. The nymphs were dissected and their haemocoel was exposed. Melanized black nodules were counted under a stereomicroscope at X60. The nodules were distinct, and direct counting

reliably reflected the extent of the nodulation response to injections (Miller & Stanley, 1998). After the first counting, the alimentary canal was removed. Nodules in the previously unexposed areas and remaining internal tissues were then counted and the two counts were summed (Miller *et al.*, 1999).

Inhibition of haemocyte micro-aggregation and nodulation

1. Influence of dexamethasone and nematodes

1.1. Dose-response

50 individuals in five groups of *S. gregaria* 5th instar nymphs were injected with 2µl of 95% ethanol or with 4µg; $4x10^{-1}$µg; $4x10^{-2}$µg or $4x10^{-4}$µg of dexamethasone in 2µl of 95% ethanol, and then co-injected with standard dosage (5 IJs / nymph) of the nematode, *H. indica* (RM_1). At 12h post-injection, the nymphs were anesthetized on ice for 10 min, then micro-aggregation and nodulation numbers were assessed as described before (Miller *et al.*, 1999). Each group experiment was repeated three times.

1.2. Time-course

Individuals in three groups of 5th instar nymphs of *S. gregaria* were injected with either 2µl of 95% ethanol as a control group, or with a standard dose of 4µg of the PLA_2 inhibitor, dexamethasone [(11β, 16α) -9-fluoro -11, 17, 21-trihydroxy -16-methylpregna-1, 4-dione] dissolved in 2µl of 95% ethanol and then nymphs were co-injected with 5IJs of *H. indica* (RM_1), the second group. The third group of nymphs was injected with 5IJs of nematode suspension only. Control and experimental nymphs were anesthetized on ice for 10 min. Number of haemocyte micro-aggregation and nodulation at each time point were then assessed at 3-10 min and at 1, 2, 6, 12, 18 and 24h post-injection according to the methods described by Miller *et al.* (1999). Each experiment was repeated three times.

2. Comparison of the influence of some eicosanoid biosynthesis inhibitors

Individuals in five groups of *S. gregaria* 5th instar nymphs were injected with either one of the cyclooxygenase inhibitors, indomethacin or aspirin. The dual cyclooxygenase /lipoxygenase inhibitor, phenidone, or the phospholipase A_2 inhibitor, dexamethasone, each inhibitor is dissolved in 2µl of 95% ethanol. Control nymphs were injected with 2µl of 95% ethanol. Immediately after injection of the inhibitors, the nymphs were further injected with the nematode *H. indica* (RM_1) infective juvenils (5IJs/nymph) as described before. 12h post- injection, the nymphs were anesthetized on ice for 10 min then haemocyte micro-aggregation and nodulation were assessed separately according to the methods described by Miller & Stanley (2001). Experiments were repeated three times each.

Control experiments

Because the insects used in these experiments were reared under semi-sterile conditions, it was expected that untreated insects would have few nodules. To determine background nodulations, 30 fifth-instar nymphs of *S. gregaria* were taken from the culture at various times throughout this work (intact control nymphs). The nymphs were anesthetized on ice for 10 min and nodule formation was assessed. To investigate the possibility that the nematode suspension of *H. indica* (RM_1) could stimulate nodulation, nymphs were treated by intrahaemocoelic injection of a standard dose of 5 IJs suspended in 2 µl distilled water and nodulation was assessed 12h later. To detect the effect of dexamethasone on nodulation in unchallenged nymphs, a standard dosage of dexamethasone of 4µg in 2 µl 95% ethanol was injected into 12 nymphs, and nodulation number was assessed 12h later. To appraise the influence of injection wounds on nodule formation, 12 nymphs were injected with 2µl of 95% ethanol (the drug vehicle) and nodulation number was then assessed 12h later following routine protocol of Miller *et al.* (1996).

Fatty acid rescue experiment

The fatty acid rescue experiment was conducted to detect the reverse effect of dexamethasone inhibition on microaggregation and nodulation. Individuals in two groups of *S. gregaria* 5th instar nymphs were injected with either 2 µl of 95% ethanol or a standard dose (4µg) of dexamethasone in 2µl of 95% ethanol, and then co-injected with 5 IJs/nymph of the nematode *H. indica* (RM_1). Immediately after injection, the dexamethasone-nematode- treated nymphs were divided into two sub-groups. Individuals in the first sub-group (about 18 nymphs) were then co-injected with 20µg of the unsaturated fatty acid, arachidonic acid [C_{20}: 4n-6 (5, 8, 11, 14-eicosatetraenoic acid)] (Sigma Chemical Co., St Louis, MO) in 2µl of 95% ethanol. Individuals in the second sub-group were similarly further treated with 2µl of 95% ethanol to control for the effects of the extra injection on nodulation. Twelve hours post-injection the nymphs were anesthetized on ice for 10 min then micro-aggregation and nodule formations were assessed separately as described before (Miller *et al.*, 1996). Experiments were repeated threetimeseach

Determination of phospholipid fatty acid composition

The insects must have the necessary precursor fatty acids and necessary enzymes for biosynthesis of eicosanoids; therefore we determined the fatty acid composition of phospholipids prepared from five tissues and one body segment: mid gut, hind gut, Malpighian tubules, cuticle, fat bodies and the head, following the protocol described by Miller *et al.* (1996) with some modifications. Individuals in three groups of *S. gregaria* 5th instar nymphs were investigated. The first group was injected with 2µl of 95% ethanol as a control. The second group was injected with 4µg of dexamethasone in 2µl of 95% ethanol and then challenged with 5IJs of the nematode *H. indica* (RM_1) suspension as described before. The third group of nymphs was injected with [4µg dexamethasone (in 2µl 95% ethanol) + 5IJs nematode] and further co-injected with 20µg of arachidonic acid in 2µl of 95% ethanol. Nymph individuals were anesthetized by chilling on ice for 10 min then a single tissue was prepared from each nymph. A total of 150 nymphs were used to prepare 5 replicate analyses of each of the six tissues. Total lipids were extracted and phospholipids were isolated as described by Howard *et al.* (1992). The samples were homogenized in 2ml of chloroform: methanol (2:1, v/v), amended with 50µl of 2% butylated hydroxytoluene to minimize autoxidation of polyunsaturated fatty acids. Total lipid extracts were applied to thin layer chromatography plates (20 x 20 cm, 250 µ Silica Gel G; Sigma Chemical Co., St Louis, MO). The plates were developed in petroleum ether: diethyl ether: acetic acid (80: 20: 1, v/v). The phospholipids remain at the origin in this chromatographic system. The silica gel bands containing the phospholipids were scraped into 15 ml screw cap reaction tubes, and fatty acid methyl esters were formed by refluxing in acidified methanol for 90 min. Fatty acid methyl esters were extracted from the reaction tubes in hexane, concentrated and analyzed by gas chromatography and gas chromatography-mass spectrometry (Miller *et al.*, 1996). The fatty acid methyl esters were chromatographed on a Hewlett-Packard 5890 gas chromatograph equipped with a Supelco wax 10 capillary column (30m x 0.25 mm Supelco Inc., Bellefonte, PA), a flame ionization detector (Varian, USA) and a Hewlett-Packard 3390 A recording integrator. The analyses were conducted by temperature programming at 2°C/ min from 150 to 240°C, using helium as carrier gas at 0.6 ml/min. Individual components were tentatively identified by comparing retention times of the components to retention times of authentic standards (Sigma Chemical Co.).

Fatty acid identifications were confirmed by gas chromatography-mass spectrometry. The fatty acid methyl esters were analyzed on a Hewlett-Packard 5890 gas chromatograph interfaced with a Hewlett-Packard 5971 electron impact mass selective detector operated at 70 eV. Separations were performed on a Supelco wax 10 capillary columns (30m x 0.25mm; Supelco, Inc.) programmed at 1°C per min from 170 to 220°C. Chromatographic conditions included (45-S splitless injection) a 2 min initial hold period, and the use of ultrapure helium as the carrier gas at 1 ml/min. Retention times and total ion mass spectra of fatty acid methyl esters were compared with authentic standards from Sigma Chemical or by comparison to published electron impact mass spectra (McCloskey, 1970)

Statistical analysis

Significant treatment effects were identified by one way ANOVA, computer program, copyright©, 1989 (H. S. Motulsky, version 1.0, Dr Schouest) UC Riverside, and serial # 8901685).

RESULTS AND DISCUSSION

Susceptibility of *S. gregaria* nymphs to the injection with the nematode, *H. indica* (RM1)

Dose-response

Assessment of the effects of varying doses of the nematode *H. indica* (RM1) isolate on *S. gregaria* nymphs to investigate a suitable dose for the immunogenic parameters studied is depicted in Table (1). Statistical analysis of the data revealed that, the LD_{25} values were 20, 5, 3 and 2 IJs/nymph at 6, 12, 24 and 36h post-injections, respectively. Insignificant differences ($P > 0.05$) were obtained in comparing the LD_{25} values at 12, 24 & 36h post-injections. Insignificant differences ($P > 0.05$) were obtained among the LD_{50} values at 12, 24 and 36h post-injection. Whereas, significant differences ($P < 0.05$) were obtained between the LD_{50} value for 6h post-injection and those values of 12, 24 and 36h post-injection.

Additionally, the nematode infective juveniles induced percentage mortality of *S. gregaria* nymphs reaching 100% at 48h post- injection with the low doses of 3 & 5 IJs/nymph. Whereas, at the higher doses (10, 15 & 20 IJs/nymph), 100% mortality was detected at 24h post- injection. The cadavers were characterized with the obvious symptoms of reddish brown coloration and melanized soft cuticle. The present work indicated that the importance of the symbiotic interaction in the pathogenesis process is clearly

Table (1): Mean mortality percentage ± S.E of *S. gregaria* nymphs at different doses and at different times post- injection with *H. indica* (RM1) nematode.

(IJs / Dose nymph)	% Mortality / hours				
	6 h	12 h	24 h	36 h	48 h
3	0^{a}	11 ± 0.57^{b}	28 ± 0.40^{c}	54 ± 0.70^{d}	100 ± 0.00^{e}
5	0^{a}	20 ± 1.45^{b}	87 ± 0.57^{c}	91 ± 0.05^{c}	100 ± 0.00^{d}
10	10 ± 2.40^{a}	50 ± 0.61^{b}	100 ± 0.00^{c}	—	—
15	20 ± 0.88^{a}	79 ± 0.84^{b}	100 ± 0.00^{c}	—	—
20	23 ± 1.20^{a}	82 ± 0.90^{b}	100 ± 0.00^{c}	—	—
Control	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

Values with the same letters in the same row are insignificant (P > 0.05).

seen in *S. gregaria* nymphs that were injected with different doses of the nematode *H. indica* (RM1) for suggesting a suitable dose for the immunogenic parameters studied.

Inhibition of haemocyte micro-aggregation and nodulation

1. Influence of dexamethasone and nematodes

Dose-response curve

The relationship between dexamethasone dosages and the number of micro-aggregates (m) and nodulations in response to the injection of the nematode *H. indica* (RM1) into *S. gregaria* 5th instar nymphs are presented in Fig. (1a & b). Increasing dosages of dexamethasone were associated with the decreased numbers of the observed micro-aggregates in response to the nematode injection. They decreased from about 149.14 x 10^{-4} m/ml haemolymph at the dosage of 4x 10^{-4} µg dexamethasone to 120.71 x10^{-4} m/ml haemolymph with the dosage of $4x10^{-2}$µg dexamethasone to approximately 69.27 x 10^{-4} m/ml haemolymph with the dosage of $4x10^{-1}$µg dexamethasone and 36.4 x 10^{-4} m/ml haemolymph with dosage of 4µg dexamethasone at 12h post- injection (Fig. 1 a) .

From Fig (1b) it is clear that the increase in dexamethasone dosages strongly suppressed nodule formation as indicated by the significant (P < 0.05) marked sharp reduction in nodule formation from 313.3 nodules/nymph at dexamethasone dosage of $4x10^{-4}$ µg to 185 nodules/nymph at dexamethasone dosage of $4x10^{-2}$µg and continued its decrease to 136.7 nodules/nymph at the dose of $4x10^{-1}$ µg dexamethasone and then reached 85.3 nodules/nymph after using the dosage of 4µg dexamethasone.

The present results revealed that the injection of different doses of the specific PLA2 pathway inhibitor, dexamethasone in *S. gregaria* nymphs pre-injected with five IJs of *H. indica* (RM1) induced increased host mortality rate as compared to the control nymphs (intact) or those injected with the carrier (ethanol) alone. This could be attributed to the fact that dexamethasone effectively retarded micro-aggregation and nodulation which evoked the increased host susceptibility towards nematode injection.

The impairment effect of dexamethasone on nodulation and aggregation reactions was evidently recorded in a dose-dependent manner. This finding supports the idea that, eicosanoids modulate micro-aggregation and nodulation reactions of *S. gregaria* haemolymph against injected juvenils of *H. indica* (RM1). This demonstration showed that the virulence of the entomopathogenic nematode can be enhanced by compromising the insect host's immune system. Similar results were also obtained by Miller *et al.* (1996) and Bedick *et al.* (2000) concerning the impairment of nodulation and micro-aggregation reaction in *Z. atratus* larvae towards infection with the bacteria *Serratia marcescens*. Also Park & Kim (2000) have detected that, different doses of dexamethasone enhanced the insecticidal effect of the symbiotic bacteria *X. nematophilus* in *Spodoptera exigua* larvae. This synergistic effect was also found to be dose-dependent. Additionally Dean *et al.* (2002) using the pathogenic fungus, *Metarhizium anisopliae* reported that dexamethasone evoked the susceptibility of *Manduca sexta* towards this pathogenic fungus.

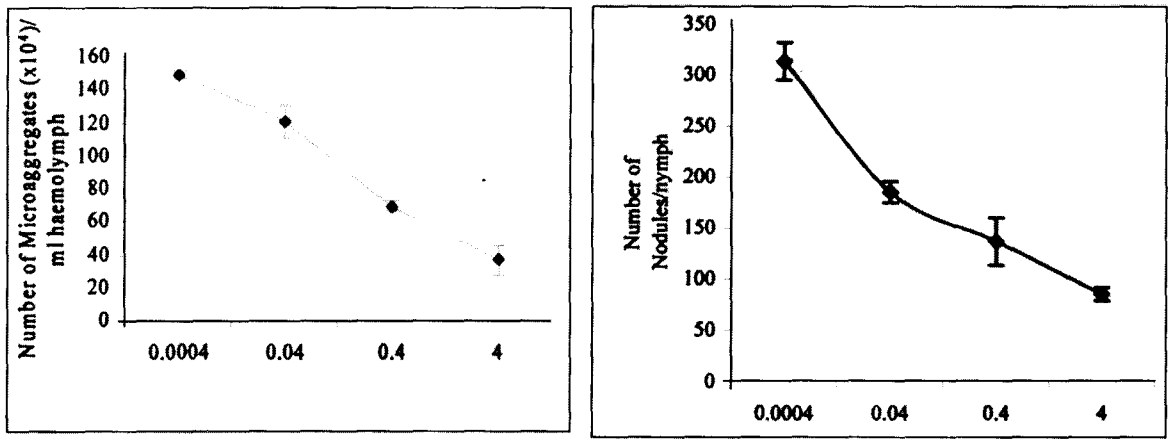
1.2. Time-course

The time course of haemocyte micro-aggregation (m) in three groups of 5th instar nymphs of *S. gregaria* is displayed in Fig. (2 a). The ethanol-treated (control) nymphs produced a moderate change in haemocyte micro-aggregation at each time point, being about 1.31 x 10^{-4}m/ml haemolymph at 3-10 min post- injection, 7.48 x 10^{-4}m/ml haemolymph at 2h post- injection, and increased to about $16.55x10^{-4}$m /ml haemolymph at 6h post-injection. The haemocyte micro-aggregation continued its significant increase (P < 0.05) during the following post- injection hours reaching a maximum of 34.48 $x10^{-4}$m /ml haemolymph at 12h post- injection.

Table (2): Fatty acids composition as percentages of total fatty acids, of phospholipids prepared from 5 tissues and head of *Schistocerca gregaria* nymphs post-injected by dexamethasone and/or arachidonic acid.

Fatty	Fat body			Head			Cuticle			Malpighian tubule			Midgut			Hindgut		
acid	Control	Dex.+N	(Dex.+N) + AA.	Control	Dex.+N	(Dex.+N) + AA.	Control	Dex.+N	(Dex.+N) + AA.	Control	Dex.+N	(Dex.+N) + AA.	Control	Dex.+N	(Dex.+N) + AA.	Control	Dex.+N	(Dex.+N) + AA.
14:01	2.63 (.15)	3.53 (.17)	3.88 (.10)	2.75 (.21)	3.42 (.14)	3.53 (.12)	2.81 (.17)	3.62 (.15)	3.78 (.13)	2.59 (.10)	3.34 (.14)	3.74 (.16)	2.71 (.17)	3.74 (.12)	3.13 (0.08)	2.30 (.74)	2.83 (.19)	4.60 (.26)
15:00	0.37 (.12)	2.72 (1.07)	3.00 (.28)	0.33 (.07)	0.48 (.10)	2.5 (.23)	0.29 (.10)	0.37 (.12)	1.93 (.90)	0.45 (.15)	0.58 (.10)	2.97 (.13)	0.31 (.07)	0.45 (.17)	3.80 (.19)	0.31 (.11)	3.5 (.23)	3.51 (.17)
16:00	12.08 (.19)	6.80 (.71)	6.20 (.06)	12.65 (.14)	9.52 (.23)	8.02 (.12)	14.70 (.17)	6.94 (.14)	7.90 (.23)	11.31 (.56)	8.51 (.14)	8.43 (.14)	13.25 (.10)	8.28 (.10)	6.32 (.13)	12.05 (.12)	7.13 (.13)	5.31 (.17)
16:1A	11.78(.45)	7.23 (.57)	6.68 (.43)	13.25 (.09)	8.39 (.12)	6.74 (.12)	14.55 (.07)	8.88 (.14)	7.40 (.47)	11.81 (.17)	7.52 (.15)	6.55 (.65)	11.78 (.23)	9.50 (.17)	6.97 (.23)	11.93 (.10)	8.55 (.07)	6.07 (.12)
16:1B	4.83 (.64)	5.13 (.30)	4.69 (.22)	3.30 (.17)	6.27 (.09)	6.56 (.10)	5.81 (.11)	7.51 (.14)	4.83 (.23)	3.55 (.65)	4.58 (.47)	6.28 (.47)	3.71 (.17)	4.49 (.17)	5.9 (.17)	4.34 (.16)	6.15 (.49)	5.15 (.49)
16:02	0.89 (.13)	2.91 (.22)	3.19 (.17)	0.81 (.17)	1.16 (.51)	2.3 (.23)	0.99 (.14)	1.28 (.11)	2.50 (.13)	0.75 (.17)	0.97 (.13)	2.82 (.10)	0.77 (.13)	1.09 (.07)	1.44 (.60)	0.81 (.19)	3.01 (.12)	3.91 (.29)
17:00	2.31 (.18)	2.87 (.23)	3.24 (.15)	2.43 (.15)	2.20 (.11)	3.13 (.12)	0.99 (.14)	1.28 (.11)	2.50 (.13)	2.15 (.49)	2.77 (.14)	3.30 (.14)	2.02 (.12)	3.30 (.17)	2.82 (.27)	2.32 (.51)	3.34 (.16)	3.28 (.53)
17:01	0.36 (.13)	4.96 (.73)	5.96 (.27)	0.43 (.12)	4.31 (.14)	5.18 (.51)	0.31 (.14)	2.98 (.14)	5.06 (.25)	0.49 (.14)	4.71 (.17)	5.73 (.12)	0.32 (.13)	3.61 (.17)	5.47 (.14)	0.53 (.17)	5.82 (.19)	5.37 (.13)
18:01	10.89(.13)	14.69 (.61)	15.10 (1.13)	13.22 (.12)	12.58 (.13)	14.81 (.26)	10.28 (.11)	13.26 (.10)	15.70 (.17)	12.09 (.25)	12.52 (.15)	14.05 (.10)	12.69 (.17)	12.57 (.65)	15.08 (.25)	11.42 (.56)	13.4 (.74)	14.33 (.11)
18:02	50.25(.12)	31.08 (.25)	30.87 (.80)	47.51 (.42)	29.18 (.55)	31.39 (.31)	45.07 (.25)	26.96 (.13)	30.36 (.44)	51.22 (.65)	30.88 (.15)	27.02 (.10)	48.68 (.64)	30.69 (.07)	30.77 (.15)	50.5 (.16)	28.15 (.49)	33.33 (.16)
18:03	1.29(.22)	6.24 (1.02)	4.95 (.24)	1.05 (.25)	8.61 (.14)	4.75 (.21)	0.84 (.13)	10.89 (.13)	4.62 (.27)	1.19 (.49)	9.38 (.50)	4.03 (.10)	0.91 (.11)	8.22 (.10)	4.75 (.21)	1.29 (.08)	3.36 (.13)	5.03 (.12)
19:00	1.20(.87)	6.64 (.82)	5.77 (.29)	0.22 (.11)	8.55 (.11)	6.05 (.12)	1.18 (.54)	8.71 (.17)	4.82 (.45)	0.34 (.14)	9.65 (.65)	5.2 (.15)	0.52 (.26)	9.06 (.12)	7.59 (.10)	0.37 (.12)	5.13 (.12)	5.73 (.16)
20:00	1.83(.10)	2.79 (.18)	3.19 (.16)	1.68 (.13)	2.38 (.10)	2.49 (.30)	1.94 (.13)	3.62 (.72)	6.33 (.14)	1.55 (.10)	2.32 (.14)	7.08 (.25)	1.44 (.60)	2.28 (.10)	2.28 (.53)	1.67 (.65)	6.15 (.49)	3.15 (1.11)
22:00	0.57 (.21)	2.71 (.23)	3.26 (.16)	0.37 (.12)	2.95 (.14)	2.55 (.07)	0.35 (.14)	2.55 (.07)	2.03 (.25)	1.51 (.15)	2.26 (.10)	2.91 (.17)	0.37 (.13)	2.68 (.64)	2.66 (.13)	0.55 (.17)	3.46 (.15)	2.52 (.13)
20:3n-6	<0.02	<0.09	<0.09	<0.02	<0.09	<0.09	<0.02	<0.09	<0.09	<0.02	<0.09	<0.09	<0.02	<0.09	<0.09	<0.02	< 0.09	< 0.09
20:4n-6	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace
20:3n-3	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace
20:5n-3	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace

Test nymphs were injected with 2µl of ethanol as a control group, the second group (Dex.+N) were injected with 4µg dexamethasone pre injected with 5 IJs/nymph of the nematode H. indicus (RM1) and the third group [(Dex.+N) + AA] were injected with 4µg dexamethasone pre-injected with 5 IJs of the nematode & co-injected with 20µg of arachidonic acid. The experiment was conducted 12h post injection. Values are mean percentages (1SEM), N = 5 separate analysis.



(a) (b)

Fig. (1): Dose-response curve for dexamethasone microaggregation (a) and nodule formation (b) of *S. gregaria* nymphs.

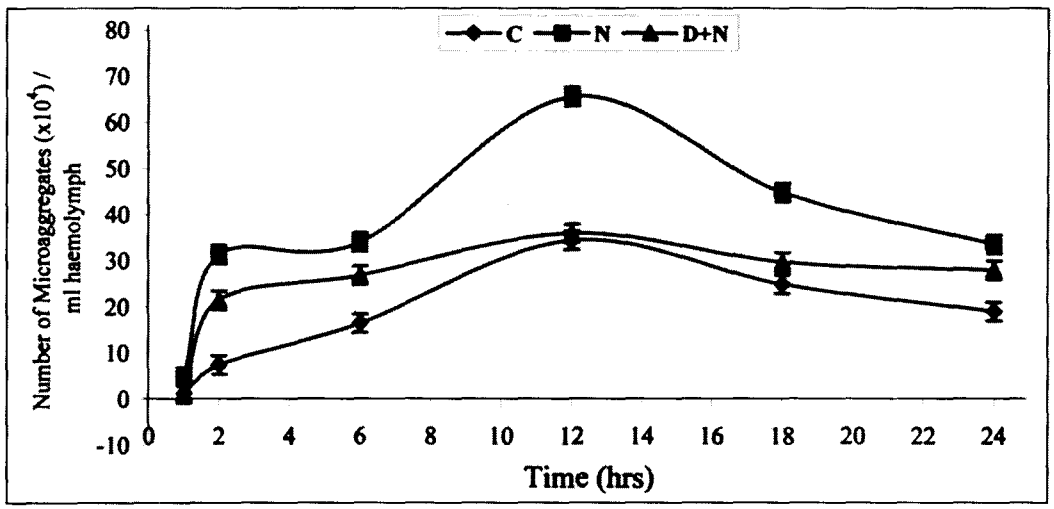


Fig. (2a). Time-course curve for *S. gregaria* nymphs injected with nematode (N), dexamethasone-treated nymphs pre-injected with nematode (D+N) and control nymphs injected with ethanol (C).

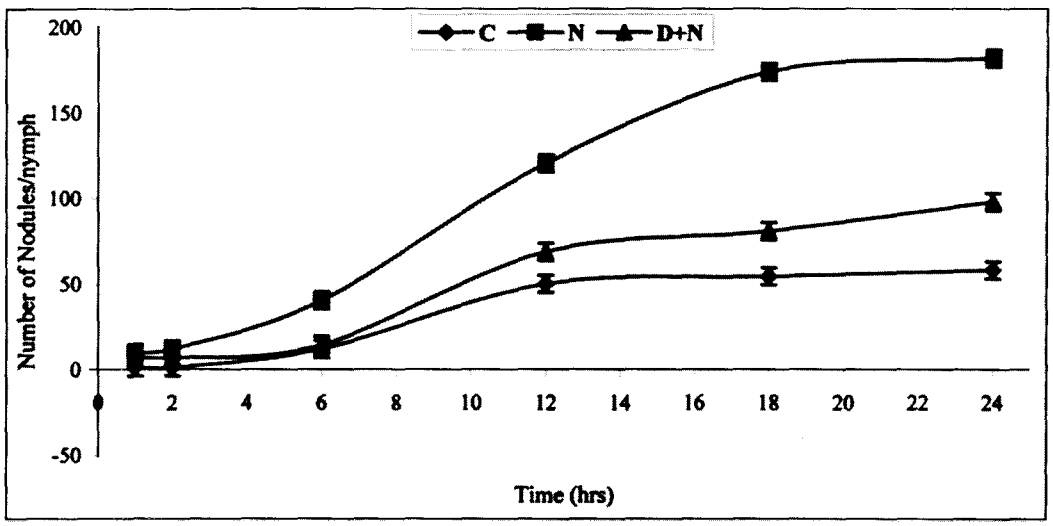


Fig. (2b). Time-course curve for *S. gregaria* nymphs injected with nematodes *H. indica* (RM1) (N), dexamethasone-treated nymphs pre-injected with nematode (D+N) and control nymphs injected with ethanol (C).

At 18 and 24h post- injection, it decreased to about 24.95 x 10^{-4} and 19.08 x 10^{-4} m/ml haemolymph, respectively. Comparable results to the ethanol-treated (control) nymphs were obtained with the nematode-injected nymphs. The haemocytic micro-aggregation increased significantly by increasing the post-injection period reaching a maximum value of 65.63 x10^{-4} m/ml haemolymph at 12h post- injection ($P < 0.05$). They declined being 44.86 x10^{-4} and 33.70 x 10^{-4} m/ml haemolymph at 18 and 24 h post- injection, respectively (Fig. 2 a)

In case of dexamethasone-nematode-treated nymphs, the number of haemocyte micro-aggregates insignificantly ($P > 0.05$) changed during the first few minutes (3-10 min) post -injection and continued in the same pattern throughout the next 24h post-injection in comparison with the ethanol-treated nymphs. Whereas haemocytic micro-aggregates obtained from the dexamethasone-nematode-treated nymphs decreased significantly ($P < 0.05$) in comparison with that of the nematode injected nymphs at each time point, being about 1.25 x10^{-4} m/ml haemolymph at 3-10 min post- injection nearly 21.52 x10^{-4} m/ml haemolymph at 2h post- injection, about 26.92x10^{-4} m/ml haemolymph at 6h post- injection, and 36.0x10^{-4} m /ml haemolymph at 12h post- injection. They then decreased reaching 29.75 x10^{-4} m/ml haemolymph and 28.02 x10^{-4} m/ml haemolymph at 18&24 h post- injection respectively (Fig.2 a).

Additionally, fig. (2 b) displays the time course of visible nodulation in each of the three groups of nymphs. The ethanol-treated (control) nymphs produced fewer than two nodules/nymph at the first and the second hours post-injections, which increased significantly ($P< 0.05$) to 12, 50, 54 and 58 nodules/ nymph at 6, 12, 18 and 24h post-injection, respectively. Whereas the nematode-treated nymphs showed significant increase in nodules than the control nymphs, reaching about 10 and 12 nodules/ nymph in the first and the second hours post-injection, respectively ($P < 0.05$). Also the nematode-treated nymphs uniformly yielded about 3-fold increase in nodulation at 6h post-injection ($P< 0.05$), in comparison with the ethanol-treated (control) nymphs. Moreover about 120 nodules/ nymph at 12 h post-injection were obtained, that significantly ($P < 0.05$) increased to approximately 174 and 182 nodules/nymphs at 18 and 24 h post-injection, respectively.

On the other hand, dexamethasone-nematode-treated nymphs showed a moderate increase in the number of formed nodules at the 1st and 2nd hour's post-injections than the ethanol-treated (control) nymphs. There were significant decrease ($P < 0.05$) between the dexamethasone- nematode-treated nymphs and the nematode-treated nymphs at each time point post-injection. By increasing the post-injection periods from 6-24h post-injection, insignificant differences in the number of nodules were obtained in comparison with the ethanol-treated (control) nymphs (Fig. 2b). Time course experiments presented in this work illustrate the rapid onset of cellular reactions, specifically micro-aggregation and nodule formation, to the nematode *H. indica* (RM1) challenge. A steep increase in micro-aggregation and nodulation reactions in *S. gregaria* nymphs during the first few minutes of nematode injection was recorded. Also, the present work indicated that co-injection with the PLA2 inhibitor, dexamethasone significantly reduced the formation of micro-aggregates and nodulation reaction in *S. gregaria* nymphs during the first hours of injection, from which we eventually proposed that eicosanoids may mediate the early phases of nodulation. Coincided results were conveniently observed by Miller *et al.* (1994 & 1996), Miller & Stanley (2001) and Dean *et al.* (2002) who recorded that, dexamethasone reduced the formation of haemocyte micro-aggregation in vitro, and the inhibitory effect of the PLA2 inhibitor, dexamethasone, on nodulation was also apparent during the first hour of infection. Stanley-Samuelson *et al.* (1991) and Horohov & Dunn (1983) have also reported that, nodulation is an early cellular reaction responsible for clearing large number of bacterial cells from the haemolymph circulation of *M. sexta* during the first 2h of infection with the bacteria *Pseudomonas aeruginosa*. The increase in haemocyte microaggregates and nodule formation at 12 and 18h post-injections of the nematode *H. indica* (RM1) in *S. gregaria* nymphs, is similar to the finding of Horohov & Dunn (1982) who showed that, humoral reactions involve induced biosynthesis of antibacterial proteins and enzymes, which appear in the haemolymph of infected insects approximately 6-12h post-infection of *M. sexta* larvae with certain pathogenic bacteria. Coincided results were also reported by Stanley-Samuelson *et al.* (1991) who proved that, the inhibition of the eicosanoid biosynthesis, using certain eicosanoid biosynthesis inhibitors, reduced the clearance of the pathogenic bacteria, *Serratia marcescens* from the haemolymph of *M. sexta* larvae resulting in an increased mortality percentage.

2. Comparison of the influence of some eicosanoid biosynthesis inhibitors

Insignificant comparable differences among the effects of the used individual inhibitors on the micro-aggregation, in comparison to the ethanol-treated control insects is presented in Fig. (3 a). On the

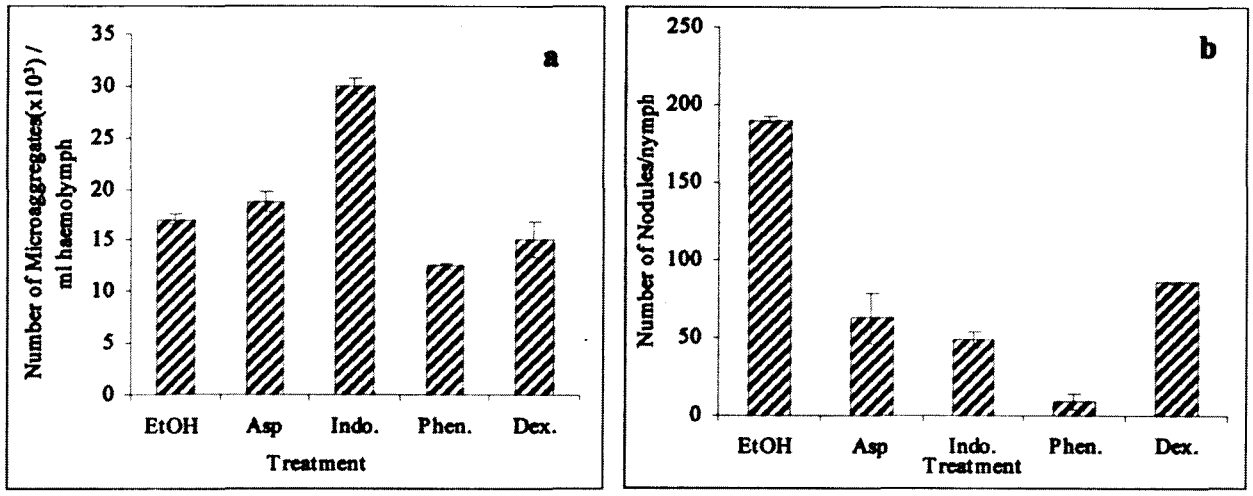


Fig.(3a & b). Influence of different eicosanoid biosynthesis inhibitors dexamethasone (Dex), aspirin (Asp), indomethacin (Indo) or phenidone (Phen) injected in *S. gregaria* nymphs injected with 5IJs of *H. indica* (RM1) nematode suspension and Control insects treated with ethanol (EtOH).

other hand, all the tested nymphs injected separately with the different studied inhibitors, exhibited significant reduction in the number of nodulation in response to the nematode injections ($P < 0.05$) (Fig.3 b). Interestingly, the dual cyclooxygenase and lipoxygenase inhibitor, phenidone recorded a very sharp significant decrease in nodule formation by about twenty folds than the ethanol-treated control. The cyclooxygenase inhibitors, indomethacin and aspirin, separately showed a moderate reduction in nodule formation by about 3 folds compared to the ethanol-treated nymphs. The phospholypase A2 inhibitor, dexamethasone appeared to be the least significant inhibitor.

Further experiments were conducted using certain inhibitors (other than dexamethasone) which are commonly used in human medicine as analgesic drugs for relief of minor pains. These compounds exert different inhibiting actions in cellular immunity (Bedick *et al.*, 2001). The present work dealing with the effect of some of these compounds on the nodulation reaction revealed that, the dual cyclooxygenase and lipoxygenase inhibitor, phenidone, recorded the highest impairment on nodulation reaction in *S. gregaria* nymphs injected with *H. indica* (RM1). The cyclooxygenase inhibitors, indomethacin and aspirin revealed comparable moderate reduction in nodule formation that was followed by the PLA2 inhibitor, dexamethasone. Similar results were obtained by Miller *et al.* (1996) on *Zophobas atratus*, Miller *et al.* (1999) on *Gryllus assimilis*, Tunaz *et al.* (1999) on *Magicicada septendecim*, Miller & Stanley (2001) on *M. sexta* and Bedick *et al.* (2001) on *Apis mellifera*. Dean *et al.* (2002) have provided additional evidence indicating that, the larvae of *M. sexta* treated with certain eicosanoid biosynthesis inhibitors with different mode of actions enhanced the susceptibility of the larvae towards the infection with the fungus, *M. anisopliae* as proved by the impairment effect of the dexamethasone inhibitor on nodulation reaction. Goldsworthy *et al.* (2003) have detected that the co-injection of the water soluble, diclofenac (another eicosanoid biosynthesis inhibitor) into *S. gregaria* and *Locusta migratoria* nymphs pre-injected with LPS extracted from *Escherichia* coli caused marked reduction in nodulation which was dose-dependent. Miller *et al.* (1999) using certain other cyclooxygenase inhibitor, naproxin, have also observed marked dose-dependent decrease of nodulation reactions formed in *Gryllus assimilis* injected with the bacteria *Serratia marcescens*. Additionally, Miller *et al.* (1994) detected that dexamethasone and certain other eicosanoid biosynthesis inhibitors inhibited nodule formation during artificial infection of *M sexta* with *Serratia marcescens*.

Our conclusion is also largely based on the effects of certain pharmacological agents that, although well characterised in mammals, are less undertaken in insects. We cannot be certain that the effects of the tested drugs are due to specific inhibition of eicosanoid biosynthesis as they may have other, non-specific effects. However, the fact that the addition of arachidonic acid reversed the immune-compromising effects of dexamethasone, as would be expected if the action of this drug was to prevent the arachidonic acid biosynthesis supports our interpretation.

Control experiments

A series of control experiments were conducted to ensure that the nodulation reactions recorded were due to the effects of injection of the nematode *H. indica* (RM1) rather than spurious responses to physical

handling and injection of other substances. Results of these experiments established background levels of nodulation in untreated (intact) 5th instar nymphs of *S. gregaria* obtained from routine culture. A mean of about 23.64 ± 05.98 nodule / nymph (n = 30 nymphs) was assessed in the untreated nymphs. The intrahaemocoelic injection with standard dosages of 5 IJs of the nematode suspension resulted in a high increase in nodule formation that reached about 292.60 ± 14.46 nodules / nymph. Dexamethasone treatments resulted in about 13.12 ± 01.09 nodules/ nymph as compared with 85.40 ± 04.34 nodules/nymph in case of injection with 2µl of ethanol, the standard carrier vehicle for all pharmaceuticals. The haemocyte reactions in insects are accomplished by different processes, from which nodulation reaction represents the quantitatively predominant major cellular defense response which is responsible for clearing large number of bacterial cells (Gillespie *et al.*, 2000). The present experiments showed that, intrahaemocoelic injection of *S. gregaria* nymphs with *H. indica* (RM1) nematodes induced a significant increase in nodule formation as compared with the ethanol-injected controls and the intact (healthy) nymphs. It was detected that, the process of nodulation was initially observed with the micro-aggregation of about 5-20 haemocytes intraping the invading bacterial cells. These micro-aggregates increased in size by continued recruitment and addition of haemocytes and bacterial cells. The overall process ends with the formation of melanized nodules containing haemocytes, bacterial cells and some haemocytes which have phagocytosed bacterial cells.

Fatty acid rescue experiment

According to the evidence taken from research in mammalian physiology, dexamethasone, as one of its actions, inhibits eicosanoid biosynthesis through its effect on the PLA2 pathway (Fig. 4). On this idea, the injection of an exogenous eicosanoid-precursor polyunsaturated fatty acid, arachidonic acid, into dexamethasone-nematode-treated nymphs was investigated on micro-aggregation (m) and nodulation reactions. Fig. (4a) shows that, the ethanol injected control nymphs yielded approximately 18.19 x 10^{-3}m/ml haemolymph that significantly ($P < 0.05$) decreased to approximately 14.62 x 10^{-3}m/ml haemolymph in nymphs injected with (4µg of dexamethasone + SIJs nematode suspension). However, nymphs injected with dexamethasone, challenged with nematode and then co-injected with the arachidonic acid yielded approximately 28.06x10^{-3}m/ml haemolymph that significantly ($P<0.05$) exceeded the ethanol (control) level. On the other hand, the control nymphs that were extra-injected with ethanol yielded approximately 12.64 x 10^{-3} m/ml haemolymph.

The arachidonic acid treatment significantly ($P<0.05$) reversed the effect of dexamethasone on nodulation reaction. The ethanol-injected (control) nymphs produced about 122 nodules/nymph that is significantly ($P < 0.05$) reduced to about 69 nodules/nymph with the dexamethasone-treated nymphs challenged with nematode suspension. Correspondingly the arachidonic acid-treated nymphs produced an obvious increased number of nodules of about 145 nodules/ nymph. The extra-injection with ethanol, yielded about 75 nodules/ nymph that are insignificantly ($P > 0.05$) differed from the dexamethasone-treated nymphs (Fig. 4 b). Fatty acid rescue experiments were also conducted, in which the exogenous eicosanoid-precursor, polyunsaturated fatty acid (arachidonic acid) was co-injected into *S. gregaria* nymphs that were injected with dexamethasone pre-injected with the nematode *H. indica* (RM1). The obtained results revealed that, the arachidonic acid reversed the inhibitory effect of dexamethasone on micro-aggregation and nodulation reactions. These reversals of experiment strengthen the evidence that eicosanoids are involved in the cellular immune

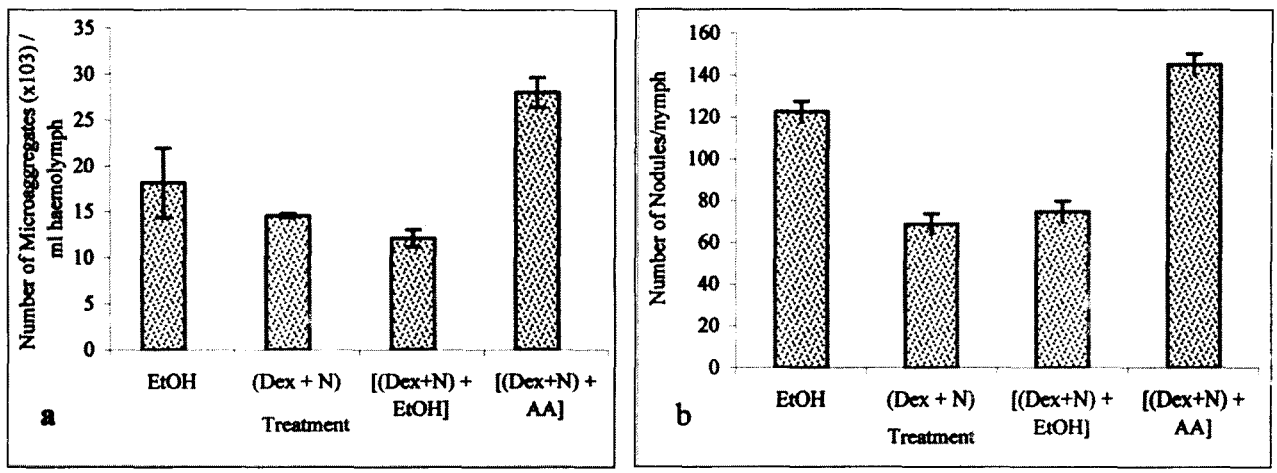


Fig. (4a & b). The eicosanoid-precursor polyunsaturated fatty acid, arachidonic acid, reversed the effect of dexamethasone on micro-aggregation (a) and nodule formation (b) in *S. gregaria* nymphs (L 5).

response of *S. gregaria* nymphs towards the nematode injections.

This work provided the basis for what is now called the eicosanoid hypothesis (formalized by Stanley, 2000) which proposed that endogenous eicosanoids mediate nodulation reactions to bacterial challenge in insects. Various authers have since used this theory and there is now much evidence that eicosanoids may mediate innate immune responses to bacteria in a wide range of insect species. However, the question of whether eicosanoids may modulate defense reactions directed against nematode injection has so far not addressed.

Fatty acid composition

Low levels of the eicosanoid precursor polyunsaturated fatty acids detected in different tissue preparations strongly support the hypothesis that micro-aggregation and nodulation reactions are specific cellular defense mechanism that is mediated by these indogenous eicosanoids. As expected from the general background of nsect fatty acid biochemistry, the even-carbon number fatty acids 16:0; 16:1A; 18:1 and 18:2 were the quantitatively major components of all tissues. The fatty acid 16:0 and 16:1A, showed a significant decrease between the control group towards the dexamethasone and arachidonic acid treatments in all insect tissues, but insignificant differences were obtained between dexamethasone and arachidonic acid treatments (Table 2).

The elaidic fatty acid 18:1 showed insignificant changes among all the three tested groups in all insect tissues (Table 2). Linoleic fatty acid, 18:2 is the quantitatively major component in all tissues that was significantly decreased with dexamethasone and arachidonic acid treatments in all tested insect tissues in comparison with the control. Whereas, it was insignificantly changed in a comparable pattern between dexamethasone and arachidonic acid. The α-lionleic fatty acid, 18:3 significantly increased with dexamethasone and arachidonic acid treatments than the control treated insect tissues. However insignificant differences were recorded between the dexamethasone and arachidonic acid treatments. The odd carbon number fatty acids 15:0, 17:0, 17:1 and 19:0 were also detected. The fatty acid 15:0 was detected in all tissues which increased significantly in arachidonic acid-treated insect tissues than dexamethasone and controls treatments, recorded its highest levels in head, cuticle, Malpighian tubules and midgut tissues. Also its level showed insignificant changes in all tissues treated with dexamethasone except in the fat body, and hind gut tissues in comparison with the control. The odd carbon number fatty acid 17:0 was insignificantly changed between the different insect tissues and the three group treatments of insect tissues (Table 2). The fatty acid 17:1, increased significantly in dexamethasone and arachidonic acid-treated nymph tissues in comparison with the control group that ranges from 10-16 folds increase with insignificant comparable pattern of both dexamethasone and arachidonic acid treatments. The fatty acid 19:0, showed a very high increase in mean percentage in all tissues treated with both dexamethasone and arachidonic acid, in comparison with the control, that was insignificantly decreased in mean percentage of all tissues between dexamethasone and arachidonic acid-treated insect tissues. The polyunsaturated fatty acids, 20:3n-6, 20:4n-6, 20:3n-3 and 20:5n-3, were present in small trace levels in all tissues. It is worth nothing that these components were detectable by mass spectrometry, but were not readily discernible by flame ionization gas chromatography.

The first step in eicosanoid biosynthesis is the hydrolysis of arachidonic acid from the cellular phospholipid pools, catalyzed by the action of the cellular phospholipase A2 (Stanley, 2000). In the present work the phospholipid fatty acids profile of *S. gregaria* nymphs obtained from various tissue sources intact, treated with dexamethasone pre-injected with the nematode *H. indica* (RM1) and/or co-injected with arachidonic acid showed moderate insignificant differences among the various treatments. The polyunsaturated fatty acids, 20:3n6, 20:4n-6, 20:3n-3 and 20:5n-3, were detected in very small trace levels. These results are similar to those of Miller *et al.* (1996) in *Z. atratus* larvae infected with the bacteria, *Serratia marcescens*. The same results were also detected by Miller *et al.* (1999) in adult *Gryllus assimilis* which is a species with many phylogentic and ecological similarities to *S. gregaria*. This finding was also supported by the idea of Stanley-Samuelson (1994) who indicated that eicosanoids are very active substances, which implies that they express their biological actions in very low concentrations.

The experiments revealed that, the co-injection of the dexamethasone with the nematode *H. indica* (RM1), strongly reduced the number of nodules compared to nematode-injected controls, and this effect was reversed by the injection of the exogenous arachidonic acid. Supported similar findings were also detected towards bacteria (Jurenka *et al.*, 1997 and Stanley-Samuelson *et al.*, 1997), bacterial LPS (Bedick *et al.*, 2000), latex microspheres (Mandato *et al.*, 1997), and most recently, to parasitoid eggs (Carton *et al.*, 2002).

However in our experiments the co-injection of low concentrations of the exogenous arachidonic acid reversed the effect of dexamethasone to some extent, the increase in the number of micro-aggregates was not statistically significant. On the other hand, Miller & Stanley (2001) and Dean *et al.* (2002) have detected a complete and significant reversal of the effects of dexamethasone with the eicosanoid- precursor arachidonic acid. There are several possible reasons for this controversy in the results including differences in the concentration of arachidonic acid used in the assay, and different microbial elicitors used as for example the nematode, *H. indica* (RM1) in the present study, the isolated bacteria *Serratia marcescens* of Miller & Stanley (2001) and the pathogenic fungus *M. anisopliae* of Dean *et al.* (2002).

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الملخص العربى

تأثير النيماتودا الممرضة للحشرات وبعض المثبطات الصيدلانية لتخليق الإيكوسانويد على حشرة الجراد الصحراوى *Schistocerca gregaria* (Forskal)

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تستهدف الدراسة الحالية تقييم حساسية العمر الخامس من الجراد الصحراوى *Schistocerca gregaria* (Forskal) للإصابة بسلالة من النيماتودا المعزولة من البيئة المصرية. قد اختبرت السلالة المصرية *Heterorhabditis indicus* (RM_1) لدراسة رد الفعل المناعى للحشرة المستهدفة لإستخدامها فى المكافحة البيولوجية. بينت الدراسة اختبار تأثير الحقن بالنيماتودا الممرضة للحشرات *H. indicus* (RM_1) تجاه العمر الخامس للجراد الصحراوى وذلك بعد تحديد الجرعة المناسبة من النيماتودا لهذا الغرض. تم اختيار الجرعة المناسبة للحقن وهى ٥ فرد من الأطوار الشابة المعدية للنيماتودا بعد ١٢ ساعة من الحقن وذلك لدراسة رد الفعل المناعى لحوريات الجراد لإنزيم الفوسفوليبيز A_2. أظهرت النتائج أن متوسط تكوين العقيدات الدموية للحشرات غير المصابة كانت ٢٣,٦٤ عقيدة لكل حشرة. وعند الحقن بـ ٥ أفراد من الأطوار الشابة للنيماتودا أظهرت النتائج الإحصائية زيادة معنوية فى تكوين العقيدات الدموية بمتوسط ٢٩٢,٦٠ فى الحشرة، والتى أظهرت نقصانا واضحا بعد حقنها بالديكساميثازون بمتوسط ١٣,١٢ عقيدة لكل حشرة مقارنة بالحشرات غير المعاملة بالديكساميثازون وقد سجل حقن الحشرات غير المصابة بمادة الإيثانول (٩٥%) متوسطا قدره ٨٥,٤٠ عقيدة لكل حشرة. أدت زيادة جرعات مادة الديكساميثازون المثبطة للعقيدات الدموية إلى نقصان عدد التجمعات الدموية كرد فعل مناعى تجاه الحقن بخمسة أفراد من النيماتودا الشابة لكل حشرة. وظهر ذلك بوضوح أثناء تكوين العقيدات الدموية. تم دراسة تأثير تغيير الوقت على التجمعات والعقيدات الدموية بعد الحقن بالديكساميثازون سجلت النتائج المتحصل عليها أن هناك زيادة معنوية فى تكوين تلك التجمعات والعقيدات الدموية أثناء الساعة الأولى للحقن واستمرت الزيادة حتى ١٢ و١٨ ساعة بعد الحقن، بينما أظهرت التكوينات للتجمعات والعقيدات الدموية نقصانا معنويا عند مقارنة حقن الحشرات المعاملة بالنيماتودا والديكساميثازون بالحشرات الأخرى المعاملة بالنيماتودا فقط. عند مقارنة تأثير حقن بعض المركبات الأخرى المثبطة لإنزيم السيكلواوكسى جينيز مثل: الإندوميثاسين والأسبرين وكذلك مركب الفينادون المثبط المزدوج لكل من إنزيمى السيكلواوكسى جينيز واللايبوكسى جينيز على تكوين التجمعات الدموية أثبتت النتائج المتحصل عليها أنه بعد مرور ١٢ ساعة من الحقن لم توجد فروق معنوية فيما بينهم مقارنة بالحوريات المحقونة بالإيثانول. كما وجد أن الحوريات المحقونة بالفينادون قد سجلت أقل معدل معنوى لتكوين العقيدات الدموية وذلك بحوالى ٢٠ مرة مقارنة بالحوريات المعاملة بالإيثانول، ثم الإندوميثاسين والإسبرين وقد أظهرت الدراسة أن مثبط الفسفوليبيز A_2 قد سجل أقل معدل معنوى لتكوين العقيدات الدموية. يتضح من تلك النتائج أن الديكساميثازون قد يكون له دور فعال فى تثبيط التجمعات والعقيدات الدموية للخلايا التى تقوم حوريات الجراد الصحراوى بتكوينها كرد فعل مناعى للحقن بالنيماتودا *H.indicus* (RM_1) لذا فإن الديكساميثازون قد يثبط من تخليق الإيكوسانويد خلال تأثيره على مسار إنزيم الفوسفوليبيز A_2. عند دراسة تأثير حقن الحمض الدهنى غير المشبع المحفز لتخليق الإيكوسانويد " arachidonic acid " على تكوين التجمعات والعقيدات الدموية فى الحوريات المحقونة مسبقاً بكل من الديكساميثازون بالاضافة إلى الأفراد الشابة من النيماتودا إلا أن: arachidonic acid كان له تأثير مساعد ومحفز لتكوين تلك التجمعات والعقيدات الدموية. وعند تقدير الأحماض الدهنية الداخلة فى تكوين الفسفوليبيدات المستخرجة من رأس الحوريات وكذلك من خمس أنسجة مختلفة من حوريات الجراد الصحراوى. قد أوضحت الدراسة أن: الأحماض الدهنية المحتوية على كربون 18:2; 18:1 16:0; 16:1A; قد سجلت أعلى المكونات فى الأنسجة المستخدمة فى الدراسة. كما وجد أن كمية الأحماض الدهنية المحتوية على كربون 16: A 16: 0 ;قد إضمحل تقديرها بصورة معنوية فى الحوريات المحقونة بالإيثانول عند مقارنتها بالحوريات المحقونة بالديكساميثازون وكذلك المحقونة بـ arachidonic acid فى جميع الأنسجة المعاملة. أما بالنسبة للاحماض الدهنية المحتوية على كربون 15:0, 17:0, 17:1, 19:0 قد إزداد فيها الحمض الدهنى 17:1 زيادة معنوية فى حالة الأنسجة المعاملة بالديكساميثازون و المعامله بـ arachidonic acid بمقارنتها بأنسجة الحوريات المحقونة بالإيثانول. أما بالنسبة للأحماض الدهنية غير المشبعة التى تعتبر المصدر الرئيسى لتخليق الإيكوسانويد وهى: 20: 3n-6; 20: 4n-6; 20:3n-3; 20:5n-3 فقد وجدت بكميات ضئيلة فى جميع أنسجة الحشرة عند إختبار تواجدها بإستخدام طريقة "mass spectrometry".