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The Role of Eicosanoids in Nodulation Reaction Against Entomopathogenic Fungi in *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) Larvae

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ABSTRACT

The study carried out the role of eicosanoids on nodulation reactions in *Spodoptera littoralis* larvae, against two fungal isolates (*Beauveria bassiana* 6646 and *Metarhizium anisopliae* 3293). When the larvae were infected with *Beauveria bassiana* 6646 and *Metarhizium anisopliae* 3293, the insects showed a nodulation reaction to this infection. Injecting eicosanoid biosynthesis inhibitors just before the *S. littoralis* larvae were infected with fungal isolates reduced the nodulation response to fungal isolates. Specific applications, including inhibitors of enzymes in eicosanoid biosynthesis; dexamethasone (a phospholipase A2 inhibitor), indomethacin, naproxen, ibuprofen (cyclooxygenase inhibitors), esculetin (a lipoxygenase inhibitor) and phenidone (both cyclooxygenase / lipoxygenase inhibitor) reduced formation of nodules in *S. littoralis* against fungal infection. These findings support the hypothesis that eicosanoids are involved in nodulation reactions against fungal infections in larvae of *S. littoralis.*

Research Article

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Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) Larvalarında Entomopatojenik Funguslara Karşı Nodülasyon Reaksiyonunda Eikosanoidlerin Rolü

ÖZET

Çalışma, iki mantar fungus (*Beauveria bassiana* 6646 ve *Metarhizium anisopliae* 3293) karşı, *Spodoptera littoralis* larvalarındaki nodülasyon reaksiyonları üzerinde eikosanoidlerin rolünü test etmiştir. Larvalar *Beauveria bassiana* 6646 *ve Metarhizium anisopliae* 3293 ile enfekte edildiğinde, böcekler bu enfeksiyona karşı nodülasyon reaksiyonu göstermiştir. *S. littoralis* larvalarına fungus izolatları ile enfekte edilmeden hemen önce eikosanoid biyosentez inhibitörlerinin enjekte edilmesi, fungus izolatlarına nodülasyon tepkisini azaltmıştır. Deksametazon (bir fosfolipaz A_2 inhibitörü), indometasin, naproksen, ibuprofen (siklooksijenaz inhibitörleri), esculetin (bir lipooksijenaz inhibitörü) ve fenidon (her ikisi de siklooksijenaz/lipooksijenaz inhibitörü) dahil olmak üzere eikosanoid biyosentezindeki enzim inhibitörleri ile yapılan spesifik uygulamalar. *S. littoralis*'te fungal enfeksiyonuna karşı nodül oluşumunun azalmasına sebep olmuştur. Bu bulgular, eikosanoidlerin *S. littoralis* larvalarındaki fungal enfeksiyonlarına karşı nodülasyon reaksiyonlarına dahil olduğu hipotezini desteklemektedir.

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INTRODUCTION

Insects develop immunity against infections in two categories, humoral and hemocytic (Gillespie et al., 1997; Rosales, 2017). Humoral immunity occurs within a few hours and they include induced synthesis of antibacterial proteins such as cecropins, attacins, diptericins and defensins (Stanley and Miller, 2006). In the presence of these proteins, bacteria lose their cellular integrity due to the detergent properties of these peptides. Insects also

secrete the lysozyme enzyme and act by hydrolyzing the cell walls of peptidoglycan bacteria (Dunn, 1986; Russell and Dunn, 1996). Hemocytic (cellular) immune reactions involve direct interactions between hemocytes in the insect circulation and infected microbes (Stanley and Miller, 2006). Cellular immune reactions include phagocytosis, nodulation and encapsulation. Nodulation is the dominant cellular defense reaction against microbial infection in insects (Stanley & Miller, 2006; Satyavathi et al.,2014). Eicosanoids are produced by enzymatic oxygenation of arachidonic acid (AA) and two other C20 polyunsaturated fatty acids. Two important groups of eicosanoids are prostaglandins and many lipoxygenase products. Almost all animals synthesize eicosanoids and eicosanides are molecular, physiologically and ecologically active in life (Stanley, 2000; Stanley & Kim, 2014). Among its important biological functions is the role of eicosanoids in insect immunity. Miller et al. (1994) reported that eicosanoids are effective in nodulation reactions created against bacterial infection in insects. Many studies have been carried out about eicosanids taking part in insect immunity until today (Stanley & Miller, 2006; Satyavathi et al.,2014, Tunaz et al., 2018). These studies show that eicosanoid biosynthesis inhibitors can inhibit the nodulation response to bacterial and other infections. This indicates that eicosanoids can play an important role in the cellular immunity of insects. In this study, the role of eicosonoids in the nodulation reaction created against entomopathogenic fungi in *Spodoptera littoralis* larvae tested.

MATERIAL and METHOD

Organisms

Spodoptera littoralis were reared on a culture (38 g agar, 2600 ml distil water, 300 g corn flour, 120 g wheat embryo, 100 g yeast, 20 g casein, 14 g wesson salt, 8 g sorbic acid, 4 g nipagin, 600 mg streptomisin, 18 g ascorbic asid and 80 mg vitamin complex) and maintained in the laboratory at 25±2 °C and 65±5% relatıve humidity (RH). The larvae (5. instars) were tested for each bioassay at 25 ± 2 °C and 65 ±5 % RH.

Entomopathogenic fungus stock cultures (isolates belonging to *Metarhizium anisopliae* and *Beauveria bassiana*) in the laboratory were used in the studies. Fungus cultures were grown in sensitive incubators in dark conditions at 25 ± 2°C and spores were obtained under these conditions. PDA and SDA media were used in the replication process. After the cultured fungus cultures completed the sporulation (approximately 4 weeks), spores were transferred to 0.02% Tween 80 solutions. After a homogeneous distribution was achieved using a shaker, its concentration was determined using a hemocytometer and the concentrations required in the experiments were reached by dilution.

All spore concentrations were prepared just before the trials and used without waiting. The germination rates of 24 hours germination rates were determined at 25 ± 2°C by cultivating 1.5 % WA medium from the sports suspensions to be used.

Reagents

The phospholipase A2 (PLA2) inhibitor dexamethasone {(11β, 16α)-9- fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-dione}, the cyclooxygenase inhibitors naproxen, indomethacin and ibuprofen {O-2-(6-methoxy-naphthyl) propionic acid}, the dual cyclooxygenase and lipoxygenase inhibitor phenidone {1-pheny-3-pryazolidinone}, and the 5- and 12-lipoxygenase inhibitor, esculetin {6,7-dihydroxycoumarin} were all purchased from Sigma Chemical Co. (St. Louis, MO).

The Role of Eicosonoids and the Effect of Eicosanoid Inhibitors

Spodoptera littoralis larvae were tested by *Beauveria*'s isolation 6646 and *Metarhizium*'s isolation 3293. The trials were organized in three replications and there were 10 individuals in each application. Eicosanoid biosynthesis inhibitors (Dexamethasone (Dex), Indomethacin (Ind), Esculetin (Esc), phenidone (Phe), Ibuprofen (Ibu) and Naproxen (Nab) were prepared as a stock culture of 0.026 µg/ml.

Spodoptera littoralis larvae were divided"into groups and each individuals in all group were injected with either the phospholipase A2 (PLA2) inhibitor dexamethasone, three of the cyclooxygenase inhibitor, naproxen, ibuprofen and indomethacin, the dual cyclooxygenase and lipoxygenase inhibitor phenidone, or the lipoxygenase inhibitor esculetin, all in standard dosages of 104 µg in 4 µl EtOH."Control insects were injected with 4 µl EtOH as control grroup. The inhibitors and control substances into the opposite side of the abdomen using a 50 µl Hamilton 701 micro-syringe were applied. Following injections, the larvae were injected with $1x10^7$ conidiaspor/ml in 5 µl 0.021 % Tween 80 solution. Application insects were incubated under the conditions of *S. littoralis* cultures and dissected under a high magnification stereomicroscope (45x) after 6 hours for nodule formation (Tunaz, 2006). Thus, whether the larvae form nodules as cellular defense against the fungus used, if so, their dimensions and the role of eicosanoids in nodule formation are revealed.

Evaluation of the Data

Nodulation data were subjected to one-way analysis of variance (ANOVA) using the SAS statistical program (SAS Ins., 1989). The differences between

the average number of nodules were revealed by applying LSD test.

RESULTS and DISCUSSION

The Role of Eicosonoids in the Nodulation Reaction of S. *littoralis* Larvae Against *Beauveria* 6646 isolate

The effects of six pharmaceutical inhibitors of eicosanoid biosynthesis on nodulation in response to fungal infections were important. As seen in Figure 1, considering the effect of six eicosanoid biosynthesis inhibitors in the nodule-forming response to fungal infection (*Beauveria*'s isolate 6646); compared to the control insects (EtOH), it significantly reduced the average number of nodules (LSD, P≤0.01). There was no significant difference between inhibitors in terms of reducing the number of nodules. Thus, it has been revealed that the larvae form nodules as cellular defense against the fungus used and eicosanoids play a role in this formation.

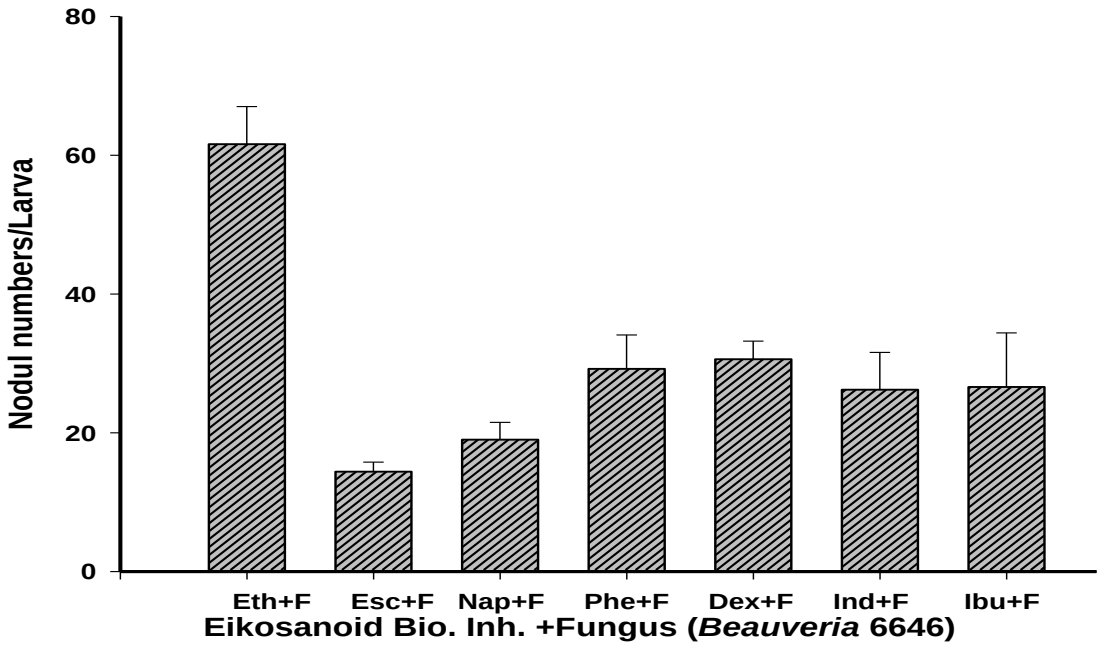

Figure 1. The effect of different "eicosanoid biosynthesis inhibitors on the nodulation reaction of *Spodoptera littoralis* larvae against fungus spores" (*Beauveria* isolate 6646).

Şekil 1. Spodoptera littoralis larvalarının fungus izolatı Beauveria 6646 karşı oluşturduğu nodülasyon reaksiyonunda farklı eikosanoid biyosentezi inhibitörlerinin etkisi.

The Role of Eicosonoids in the Nodulation Reaction of S. *Littoralis* Larvae Against *Metarhizium* 3293 isolate

As in the previous study, the effects of six eicosanoid biosynthesis pharmaceutical inhibitors on nodulation in response to fungal infections were evaluated. As seen in Figure 2, considering the effect of six eicosanoid biosynthesis inhibitors in the nodule-forming response to fungal infection (*Metarhizium* no. 3293); Esculetin inhibitor decreased nodulation formation the most when compared with control insects (EtOH) (LSD, P≤0.01).

Compared to control, all inhibitors reduced the number of nodules (LSD, P≤0.01). There were differences between inhibitors in terms of decreasing the number of nodules. Thus, it has been revealed that the insects form nodules as cellular defense against the fungus used and eicosanoids play a role in this formation.

In the study, the results supported the hypothesis that eicosanoids mediate nodule formation in response to fungal infections in *S. littoralis* larvae. "Six different eicosanoid biosynthesis inhibitors significantly reduced nodulation compared to control applications. Similarly, Tunaz et al. (2018) tested that eicosanoids mediate nodule formation in response to fungal infection in German cockroach adults. As in this study, six different eicosanoid biosynthesis inhibitors (Dexamethasone, Indomethacin, Esculetin Phenidone Ibuprofen and Naproxen) significantly reduced nodulation compared to control applications. On the other hand, Tunaz et al. (2008) tested whether eicosanoids mediate nodule formation in response to fungal (*Beauveria bassiana* HRI-215 isolate) infection in potato beetle larvae; Of the seven different eicosanoid biosynthesis inhibitors (Dexamethasone, Indomethacin, Esculetin Phenidone Ibuprofen

Piroxicam and Naproxen), only Phenidone has significantly reduced the number of nodules in the larvae compared to control applications. This study shows that eicosanoid biosynthesis inhibitors prevent nodule formation may differ from insect to insect.

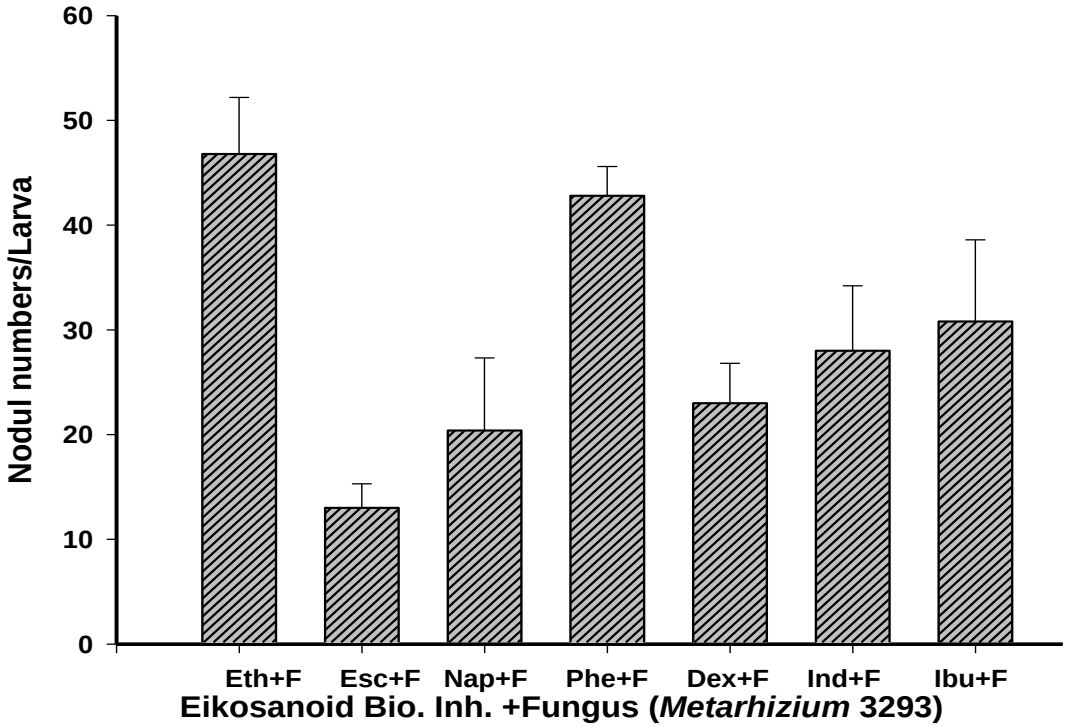

Figure 2. The effect of different"eicosanoid biosynthesis inhibitors on the nodulation reaction of *Spodoptera littoralis* larvae against fungus spores (*Metarhizium* isolate 3293)."

Şekil 2. Spodoptera littoralis larvalarının fungus izolatı Metarhizium 3293 karşı oluşturduğu nodülasyon reaksiyonunda farklı eikosanoid biyosentezi inhibitörlerinin etkisi

If examined the effects of six different eicosanoid biosynthesis inhibitors in detail in this study; the chemicals used in this study inhibit different enzymes in the path of eicosanoid biosynthesis to inhibit eicosanoid synthesis in *S. littoralis* larvae. In the eicosanid biosynthesis pathway, dexamethasone inhibits phospholipase A2 enzyme; naproxen, indomethacin, ibuprofen inhibits cycloxygenase enzyme; esculetin inhibits lipxygenase enzyme; among the inhibitors, phenidone inhibits both cyclooxygenase and lipxygenase enzyme in the biosynthesis pathway (Stanley, 2000). Based on this information, both cyclogenase and lipoxygenase synthesis path reveal the biochemical effect of eicosanides in the immunity of these insects. In addition, this study has revealed that inhibition of eicosanoids, which have recently been associated with insect pathology and thus microbial control, can suppress the insect immune system, causing an accelerated biological activity and increased mortality rates, particularly as a result of entomopathogenic fungus applications. When eicosanoid inhibitors and entomopathogenic fungus isolates (*Beauveria* isolate 6646 and *Metarhizium* 3293) were applied together to *S. littoralis* larvae, it was observed that nodule formation, which is one of the cellular immunity, so entomopathogenic fungus isolates were applied together with eicosanoid inhibitors. Co-administration of eicosanoid inhibitors with fungal isolates may have a higher mortality rate than trials without eicosanoid inhibitors.

Researchers Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

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