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New Approaches to the Control of *Neodiprion sertifer* (Geoffroy, 1785) (Hymenoptera: Diprionidae)

Neodiprion sertifer (Geoffroy, 1785) (Hymenoptera: Diprionidae)'in Mücadelesinde Yeni Yaklaşımlar

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ABSTRACT

Neodiprion sertifer (Geoffroy) (Hymenoptera: Diprionidae) is a major pest on *Pinus* spp. in Europe. In this study, new control methods were tried to control *N. sertifer*. The effectiveness of *Bauveria bassiana* (Nostalgist, Bb %1.5 1 x 10⁸ kob /ml min, 250 ml/100L), azadirachtin (Nimbecidine, 0.3g azadirachtin/l, 500 ml/da-100L; Nimiks4.5, 40g azadirachtin /l, 200 ml/100L; Neem Azal ®-T/S, 10 g azadirachtin /l, 300ml/100L) and spinosad (Oread, 480 g spinosad/l, 10ml/100L) was tested on *N. sertifer* larvae. In addition, the effect of the idiobiont parasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae) venom was evaluated. *Bauveria bassiana*, spinosad and azadirachtin were applied to the larvae by spraying method. Different numbers of *B. hebetor* females (1-5 females) were also tested on *N. sertifer* larvae. All experiments were carried out at 25 ± 1°C, 60-70% relative humidity and 16L:8D h photoperiod. After *B. bassiana* application, the survival rates were 71.66% and 61.66% at the 6th day and 7th day. The maximum efficacy was obtained with azadirachtin in Nimiks 4.5 (40 g azadirachtin/l). The highest mortality (100.0%) was found at 24 h after the application of spinosad (10ml/100l). The most effective combination of *B. hebetor* female venom on *N. sertifer* larvae is 5 females and 7 days duration. The study shows that spinosad, azadirachtin and *B. hebetor* female venom can achieve effective control of *N. sertifer* larvae.

Key Words: *Neodiprion sertifer*, *Bauveria bassiana*, spinosad, azadirachtin, *Bracon hebetor* venom

ÖZ

Neodiprion sertifer (Geoffroy) (Hymenoptera: Diprionidae) Avrupa'da *Pinus* spp.'nin en önemli zararlısı konumundadır. Bu çalışmada *N. sertifer*'in mücadelesi için yeni mücadele yöntemleri denenmiştir. *Bauveria bassiana* (Nostalgist, Bb %1.5 1 x 10⁸ kob /ml min, 250 ml/100L), azadirachtin (Nimbecidine, 0.3g azadirachtin, 500 ml/da-100L, Nimiks4.5, 40g azadirachtin /l, 200 ml/100L; Neem Azal ®-T/S, 10 g azadirachtin /l, 300ml/100L) ve spinosad'ın (Oread, 480 g spinosad/l, 10ml/100L) etkinliği *N. sertifer* larvaları üzerinde denenmiştir. Ayrıca idiobiont parazitoit *Bracon hebetor* (Say) (Hymenoptera: Braconidae)'un venomu da değerlendirilmiştir. *Bauveria bassiana*, spinosad ve azadirachtin larvalara püskürtme yoluyla uygulanmıştır. Farklı sayıdaki *B. hebetor* dişi de (1-5 dişi) *N. sertifer* üzerinde test edilmiştir. Bütün denemeler 25 ± 1°C, 60-70% orantılı nem ve 16:8 saat aydınlık:karanlık koşullarda gerçekleştirilmiştir. *Bauveria bassiana* uygulamasından sonra larvaların yaşam oranı 6. ve 7. günde %71.66 ve %61.66 olarak

bulunmuştur. Azadirachtinde maksimum etkinlik Nimiks 4.5 (40 g azadirachtin/l)'de elde edilmiştir. En yüksek ölüm oranı (%100) spinosad (10ml/100l) uygulamasından 24 saat sonra bulunmuştur. *B. hebetor* dişi venomunun *N. sertifer* larvaları üzerinde etkili olduğu kombinasyon 5 dişi ve 7 gün süredir. Bu çalışma spinosad, azadirachtin ve *B. hebetor* dişi venomunun *N. sertifer* larvaları üzerinde etkili bir kontrol sağlayabileceğini göstermiştir.

Anahtar Kelimeler: *Neodiprion sertifer*, *Bauveria bassiana*, spinosad, azadirachtin, *Bracon hebetor* venom.

Introduction

Neodiprion sertifer (Geoffroy) (Hymenoptera: Diprionidae) is a European diprionid species which is the most widely distributed in the world. It was recorded in New Jersey, USA in 1925 and in Ontario, Canada in 1939 (Lyons, 1964; Pschorn-Walcher, 1965; 1982, Griffiths et al., 1984). The larval stage feeds on the needles of pines such as *Pinus banksiana* (jack pine), *Pinus sylvestris* (Scots pine), *Pinus cembra* (Arolla pine), *Pinus contorta* (lodgepole pine) and *Pinus densiflora* (Japanese umbrella pine) (Baş, 1973; Tosun, 1975; Şimşek & Kondur, 2006; Aksu, 2010). *Neodiprion sertifer* can reach epidemic population levels in pine forests (Larsson & Tenow, 1984; Virtanen et al., 1996; Lyytikäinen-Saarenmaa & Tomppo, 2002).

Chemical insecticides have long been used for controlling *N. sertifer* population. However, they cause a many negative health and environmental problems (Yaman et al., 2001). The use of environmentally friendly control methods against *N. sertifer* have been started many years ago. It was first described by Escherich (1913) as natural nucleopolyhedrosis virus (NPV) (Baculovirus) that reduces *N. sertifer* populations. Cunningham & Entwistle (1981) noted that virus infection spreads rapidly in *N. sertifer* population via defecation of larvae. Nevertheless, due to the virus activity, the population of *N. sertifer* was decreased very rapidly. Göktürk & Tozlu (2019) applied different doses of *Bacillus thuringiensis* subsp. *kurstaki* ABTS-35 and Natural-Pyrethrum (obtained from *Chrysanthemum cinerariaefolium*) to *Diprion pini* L. (Hymenoptera: Diprionidae) and *N. sertifer* larvae. Dipel (Bt) 500g/100 l dose was found to be the most effective dose on *N. sertifer*. However, it has been reported that a dose of 600 ml of Spruzit Neu (Pyrethrum) has a significant effect on *N. sertifer* larvae.

Azadirachtin is a tetranortriterpenoid obtained

from the seed of neem tree [*Azadirachta indica* A. Juss (Meliaceae)], is very known commercialized biopesticide and the most successful botanical pesticide that is used against agricultural pests in the scope of Integrated Pest Management programmes. Azadirachtin has an antifeeding, repellent, adult sterility and insect growth regulator effect on the pest. However, it is considered that it has low residual effect and low side-effect on biocontrol agents, predators, and parasitoids (Tunca et al., 2012; Biondi et al., 2013; Abedi et al., 2014, Cura et al., 2019; Kilani-Morakchi et al., 2021).

Beauveria bassiana was first obtained from silkworm cadavers, and it is effective on more than 200 insects (Nakahara et al., 2009). *B. bassiana* produces numerous toxins (beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, oxalic acid, calcium oxalate crystals, and many beauvericin analogs) that cause infection in insects (Chelico & Khachatourians, 2008; Naqqash et al., 2016; Wang et al., 2021).

Spinosad is a neurotoxic (effective on the nervous system) insecticide. *Saccharopolyspora spinosa*, an actinomycete bacterium of soil origin, naturally produces metabolites of Spinosyn A (85%, C₄₂H₆₇NO₁₆) and Spinosyn D (15%, C₄₁H₆₅NO₁₆) as a result of fermentation under airless conditions. Spinosad has demonstrated high levels of efficacy against lepidopteran larvae, as well as some Diptera, Coleoptera, Thysanoptera, and Hymenoptera, has a range of effects on insects including as a contact, stomach poison effect, repellent, antifeedant, and growth and mating inhibitor (Anastas et al., 1999; Copping & Menn, 2000; Cisneros et al., 2002; Williams et al., 2003; Mayes et al., 2003; Isman, 2006; Matthews, 2006; Joseph, 2020).

Bracon hebetor Say (Hymenoptera: Braconidae) is a gregarious, idiobiont ectoparasitoid that attacks larvae of several

species of Lepidoptera (Brower et al., 1996; Hagstrum & Smith, 1997). *Bracon hebetor* females paralyze their host before oviposition and then may deposit a different number of eggs near or on the paralyzed hosts. Meanwhile, female parasitoid caused irreversible paralysis of the host insects. (Ghimire, 2008). There have been no studies on the effects of azadirachtin, *B. bassiana*, spinosad and *B. hebetor* venom on the *N. sertifer*. The objective of this study was to evaluate their potential efficacy for future IPM program options.

Materials and methods

Insect

Third instar larvae of *Neodiprion sertifer* were collected from Konya-Hüyük province (37.93023°Lat, 31.71091°Long) and transferred to the laboratory. Larvae were reared to the insect rearing box (25 x 35 x 17 cm) with fresh black pine needles and placed in the climate room (25 ± 1°C, RH 65 ± 5% and L : D 16 : 8 h photoperiod).

Insecticides

In our study, three biorational insecticides, *Beauveria bassiana* (Nostalgist, Bb %1.5 1 x 10⁸ kob /ml min, application of dose is 250 ml/100L), azadirachtin (Nimbecidine, 0.3g azadirachtin/l, application of dose is 500 ml/da-100L; Nimiks4.5, 40g azadirachtin /l, application of dose is 200 ml/100L; Neem Azal ®-T/S, 10 g azadirachtin /l, application of dose is 300ml/100L) and spinosad (Oread, 480 g spinosad /l, application of dose is 10ml/100L) were used. However, 1-5 females of *B. hebetor* were used to test the efficacy of *B. hebetor*'s venom.

Bioassays

For this purpose, one third instar larvae were placed in petri dishes (6.5 cm) with blotting paper. Application doses of each insecticide were applied to 20 third instar larvae using the hand spray bottle. After treatment, the larvae were placed to the climate room (25 ± 1°C, RH 65 ± 5% and L: D 16: 8 h photoperiod) and provided with black pine needles. Each treatment was carried out in triplicate with 20 larvae. A total of 60 larvae were used for one application. 660 larvae were used for 11 treatments, including control. The alive larvae were checked daily during 7 days.

Statistical analyses

Survival data was calculated as a percentage and were normalized using an arcsine transformation ($p' = \arcsin \sqrt{p}$, Zar, 1999). Transformed data were analysed using ANOVA ($\alpha=0.05$) and Tukey's mean separation ($\alpha=0.05$). Statistical analyses were carried out using MINITAB computer software Release 17.

Results and Discussion

All biorational insecticides had adverse effects on *N. sertifer* at different incubation period after the treatment in this assay (Table- 1) (df=76; F=71.65; P≤0.05). Oread (Spinosad) induced 100% mortality and it was significantly greater than all other treatments (Fig. 1). The highest mortalities of this pest were obtained with Nimiks (40 g azadirachtin /l). The mortality rate increased with the increase of incubation period (Figure 1). The survival rates of *N. sertifer* larvae were found 63.33 %, 78.33 %, 90.00 % at seventh day, in Nimiks, Neem Azal (10g azadirachtin /l) and Nimbecidine (0.3g azadirachtin/l), respectively.

Table 1. Survival rate (%) caused by different treatments to third instar *Neodiprion sertifer* larvae after 1-7 day incubation at 25±1°C.

Treatments	Days						
	1	2	3	4	5	6	7
Nostalgist 250 ml/100l	100A	100A	91.67AB	86.67BC	85BC	71.67DE	61.66EF
Nimbecidineb500ml/100l	100A	100A	98.33A	95AB	95AB	91.66AB	90 ABC
Nimiks 200 ml/100l	91.67A	86.67 BC	85.00BC	80 CD	80CD	70DE	63.33EF
Neem Azal 300ml/100l	100A	96.67 AB	93.33AB	91.66AB	88.33BC	85 BC	78.33D
Oread 10 ml /100l	0	0	0	0	0	0	0
<i>Bracon hebetor</i> (1 dişi)	96.67AB	93.33 AB	88.33BC	88.33 BC	86.66BC	86.67 BC	83.33BC
<i>Bracon hebetor</i> (2 dişi)	93.33AB	88.33 BC	88.33BC	88.33 BC	88.33 BC	83.33 BC	78.33D
<i>Bracon hebetor</i> (3 dişi)	80CD	73.33DE	71.67DE	66.67 EF	65EF	63.33EF	58.33FG
<i>Bracon hebetor</i> (4 dişi)	53.33FG	48.33GH	46.67GH	38.33HI	36.67HI	33.33 IJ	33.33IJ
<i>Bracon hebetor</i> (5 dişi)	30IJ	23.33JK	18.33KL	13.33KL	11.66KL	10L	8.33L
Control	100A	100A	100A	100A	95AB	95AB	95AB

*Means followed by a different uppercase letter are significantly different ($P < 0.05$, ANOVA, Tukey).

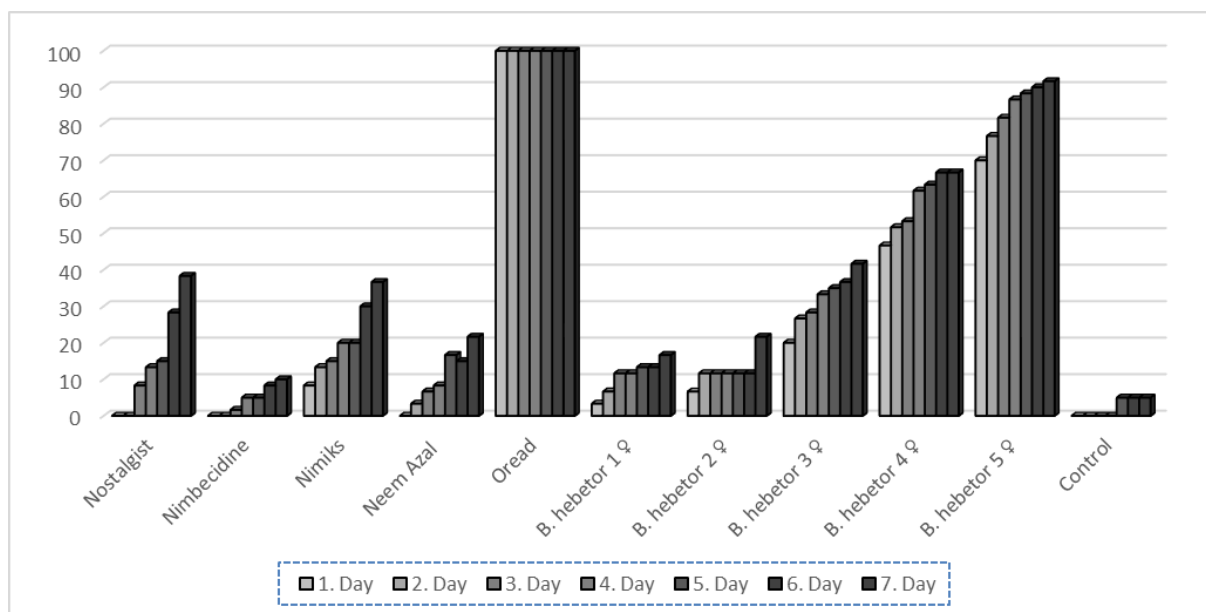


Figure 1. Percent mortality of *Neodiprion sertifer* larvae at different treatments and at 1, 2, 3, 4, 5, 6 and 7 days interval.

The mortality induced by the *B. bassiana* after 144 h of incubation. The highest mortality level (38.34%) for the *N. sertifer* larvae was recorded after 7 days with treatment of *B. bassiana* (Figure 1). *Bracon hebetor* venom had a strong effect on the survival of larvae of *N. serifer* (Table 1). The mortality rate was found significantly higher with increased of the incubation period and the number of females. Mortality rates increased

with the treatment of 4 and 5 females (Figure 1). After Oread, *B. hebetor*'s venom has been found to be most effective treatment for *N. sertifer*. However, mortality rate never exceeded 5% in the control (Figure 1).

Biorational insecticides known as “third-generation insecticides” or “reduced-risk insecticides” are derived from natural compounds and have low or no side effects on the

environment or beneficial organisms (Hara, 2000; Horowitz & Ishaaya, 2004; Kapoor & Sharma, 2020). Studies on the impact of biorational insecticides on the *N. sertifer* are limited to baculovirus and pyrethrum (Cunningham & Entwistle, 1981; Podgwaite et al., 1984, Yaman et al., 2001; Göktürk & Tozlu, 2019). In this study, the most effective result was obtained with spinosad among the tested insecticides. All larvae were dead within the first 24 hours (Table 1, Figure 1). Spinosad treatments were also toxic to *Mamestra configurata* Walker (Lepidoptera: Noctuidae), *Phyllotreta cruciferae* Goeze (Coleoptera: Chrysomelidae) (Elliot et al., 2007). Kowalska (2008) noted that all concentrations of spinosad (0.2%, 0.1% and 0.05%) caused mortality both *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) adults and larvae depending on the temperature. Maral (2021) reported that, the highest mortality rate of *Stefanitis pyri* (Fabricius) (Heteroptera: Tingidae) adults was found in spinosad after that azadirachtin and kaolin.

In this study, the effect of azadirachtin varied depending on the rate of active substance, dose and incubation period. Nimiks (40 g azadirachtin/l) was also found to be the more effective than the other azadirachtin insecticides (Table 1). It can be preferred in the control of *N. sertifer*. Kraiss & Cullen (2008) noted that two neem insecticides (neem seed oil and azadirachtin) were effective in causing nymphal mortality of *Aphis glycines* (Matsumura) (Heteroptera: Aphididae) (77% by neem oil and 80% by azadirachtin). Neem oil formulation also presented significant efficacy on *Idioscopus clypealis* (Lethierry,) (Hemiptera: Cicadellidae), a mango pest (Adnan et al., 2014). Lin et al. (2021) noted that azadirachtin was effective on *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) and *Rhopalosiphum maidis* (Fitch) (Heteroptera: Aphididae).

Beauveria bassiana is the most studied biorational insecticides. Like azadirachtin, *B. bassiana* was applied for the first time against *N. sertifer*. The highest mortality was obtained on the 7th day (38.34 %) (Table 1, Figure 1). A high

case mortality rate was not observed in *N. sertifer* larvae. Studies on *Beauveria* have focused especially on the order Lepidoptera. Wraight et al. (2010) reported that *Obstrinia nubilalis* (Hübner) (Pyralidae), *Helicoverpa zea* (Boddie) (Noctuidae) and *Spodoptera frugiperda* (Smith) (Noctuidae) (Lepidoptera) are highly susceptible of *B. bassiana* strains. Mortality of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) was %0, %8 and %10 at 24, 72 and 120 h, respectively (Tunçsoy et al., 2020). Gao et al. (2022) reported that *B. bassiana*-PfBb can be used as a bio-insecticide to control young larvae of *S. frugiperda*. *B. bassiana* (BMAUM-M6-4) isolate was caused significant infection in early and late instar larvae of pine processionary (Gök et al., 2018). In another study, it was determined that nymphs and adults of *Palomena prasina* L. (Heteroptera: Pentatomidae) were susceptible to *B. bassiana* (Yiğit and Saruhan, 2022). Besides, side effect studies were mostly conducted on parasitoids in the Hymenoptera order, such as *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) (Labbé et al., 2009), *Trichogramma cacoeciae* (March) (Hymenoptera: Trichogrammatidae) (Tunçsoy et al., 2020), *Coptera haywardi* (Oglobin) (Hymenoptera: Diapriidae) (Martínez-Barrera et al., 2020), *Trichogramma dendrolimi* (Mastumura) (Kandil, 2022). However, no study was found on a pest belonging to the order Hymenoptera.

Parasitoid venoms compose of a complex mixture of proteinaceous and non-proteinaceous components that thought as agrichemicals to improve pest management (Morreanu & Asgari, 2015). The main effect of toxin (venom of ectoparasitoid) is the influence of short and long-term paralysis in the host during the larval and nymphal stages to start developmental block. On the other hand, toxin might have different roles during induction, such as restraining the immune system or disrupting development (Edwards et al., 2006; Price et al., 2009; Tian et al., 2010; Kryukova et al., 2011).

Idiobiont parasitoid *Bracon hebetor* causes paralysis of host (Quistad et al., 1994). Three proteins were determined in the venom, two of

them (Brh-I and -II) being insecticidal when injected into lepidopteran larvae (Ferber et al., 2001). The constituents of the venom, which comprise of proteins from 3.4–200 kDa have not been well characterized (Gnatzyet al., 2000). In this study, effect of *B. hebetor*'s venom was investigated on another Hymenopter species, *N. sertifer*. It was found that *B. hebetor* paralyzed larvae of *N. sertifer* larvae but did not deposit eggs. The level of paralysis was increased by the number of parasitoid females. Mortality rates were found to be 66.67 % and 91.67%, respectively with application of 4 and 5 females at 7th day (Table1, Figure 1). Parasitoid female venom has a highly toxic effect on *N. sertifer*'s larvae.

Conclusion

As a result, spinosad and *B. hebetor* female venom can offer effective control of *N. sertifer*'s larvae and prevent serious damage to *Pinus* spp. Azadirachtin and *B. bassiana* can be used with the combination of the *B. hebetor* venom of in an integrated pest management program.

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