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The Effect of Pyrethrum and *Bacillus thuringiensis* Biopesticides on *Diprion pini* L. and *Neodiprion sertifer* (Geoffr.) (Hymenoptera: Diprionidae) Larvae

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ABSTRACT

The larvae of the common pine sawfly (*Diprion pini* L.) and the European pine sawfly (*Neodiprion sertifer*) can cause epidemic, around the globe. Recently, an outbreak has been reported in young Scots pine (*Pinus sylvestris* L.) forest in Artvin - Borçka, Turkey. In order to propose effective control measures, this study was conducted to estimate the effectiveness of two biopesticides against *D. pini* ile *N. sertifer* larvae. Different doses of Pyrethrum and *Bacillus thuringiensis* biopesticides (Spruzit® Neu from 150ml to 600ml/100 l, and DiPel® DF BT 100 to 500 g/100 l) were applied against the larvae of *D. pini* and *N. sertifer* their impact was monitored under laboratory conditions. The finding of the study revealed that the most effective dosages for larvae of *D. pini* was Dipel 300 g/100 l and Dipel 500 g/100 l of *B. thuringiensis*. The efficiency ratio of Pyrethrum doses ranged from 59.5%-78.5% for Spunizet Neu and 85.5%-95.5% for Dipel DF against *D. pini* larvae. It was observed that the dosage of both pesticides was directly propositional to the death in larvae. Intriguingly, Dipel at the dose rate of 500 g/100 l was the most effective applications for larvae of *N. sertifer*. While variable impacts were noticed against larvae, both biopesticides were effective against larvae of *D. pini* and *N. sertifer.* Taken together, finding of this study propose the use of Pyrethrum and *Bacillus thuringiensis* biopesticides to control common pine sawfly and the European pine sawfly in the event of an epidemics in Turkey.

INTRODUCTION

Turkey is an ecogeographically rich country, and 27% (20,712,894 ha) of the country-area is covered by the forests. Eastern Black Sea Region and Artvin are enriched with the natural forest and are represented by 54% and 2% of the Turkish forests, respectively. The dominant tree in the forests is Oriental Spruce, which is spread in an area of 25.628 ha. Other trees including Scotch pine, oriental beech, *Abies nordmanniana* (Steven) Spach, mountain alder, Anatolian chestnut and stone pine covers a significant proportion of the forest area (Eminağaoğlu *et al*., 2015). However, this rich forest is predisposed to several predators. Scotch pine (*Pinus sylvestris* L.) that represents an area of 217,104 hectares (5.37%) is under threat of reddish-yellow bush antenna sawfly (*Neodiprion sertifer* (Geoff.)) and bush antenna pine sawfly species (*Diprion pini* Linnaeus, 1758) (Hymenoptera: Diprionidae).

N. sertifer and *D. pini* are among the well-known coniferous tree pesticides for pine forest in the Europe (Barre *et al*., 2002; Dajoz, 2000; Turrisi and Bella, 1999). Additionally, these are reported in pine forests in Northern and Middle Europe and Asia and Northern America (Olofsson, 1987). These species cause epidemic periodically in prevalent areas leading to severe economic losses (Geri, 1988). In Turkey, both *N. sertifer* and *D. pini* species are predominantly prevalent in Mediterranean, Aegean, Marmara and Black Sea Regions, and cause damage to all pine forests (Çanakçıoğlu and Mol, 1998; Nafisi, 1999; Çuhadar *et al*., 2000; Şimşek and Kondur, 2006; Yaman *et al*., 2001; Aksu, 2010).

The damage caused by *N. sertifer* and *D. pini* is associated with eating leaves of pine trees. These damages are primarily reported in trees that are 10-15 years old. The trees attacked by *N. sertifer* and *D. pini* may become naked after all of their leaves are eaten and may be vulnerable to the attack of other pests because of their pre-existing weaknesses (Çanakçıoğlu and Mol, 1998; Romanyk and

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Cadahía, 2003; Augustaitis, 2007).

Today, silvicultural, mechanical, biological, biotechnical and chemical methods are being used as pests control strategies in forests. In Turkey, there are limited studies that detailed the extent of damage caused by *D. pini and N. sertifer* and mechanisms of control (Aksu, 2010; Akıncı and Avcı, 2016). Several methods are proposed to fight against these harmful pests, however, chemical control is one of the most frequently used methods around the globe (Demirbag *et al*., 1997). In Turkey, powder or liquid formulation of pesticides are recommended against these important sawflies (Çanakçıoğlu and Mol, 1998; Linstedt *et al*., 2006). There are several studies in which Diflubenzuron WP-25 was used against *N. sertifer* larvae in black pine plantation (Şimşek and Kondur, 2006). Additionally, it has been reported that decrease in pest population was observed after the use of the nuclear polyhedrosis virus (NsNPV) application against *N. sertifer* (Lord, 2005). Again, Anderbrant *et al*. (1998, 2000) and Östrand *et al*. (2000) reported that gonad pheromones could be used to reduce the *N. sertifer* populations and to monitor the population density.

According to the Environmental Protection Agency of the USA (EPA, 2014), there are more than 21,000 pesticides in the USA. Alternative biopesticides have been used for 30 years to synthetic insecticides and the production of synthetic chemicals has reduced at a rate of 2%, whereas the production of biopesticides has increased at a rate of 20% on annual bases (Cheng *et al*., 2010). Since chemical pesticides pose an environmental problem (Arora *et al*., 2012; Gülhane *et al*., 2015), integrated pest management (IPM), which includes biotechnical, mechanical and biological fights has become necessity rather than using the existing plant protection practices. The importance of biological fight, which is included in IPM, has increased in recent years. Applying entomopathogen organisms in biological fight has an important contribution in pest management. Among these organisms, it has been proven in many studies that *Bacillus thuringiensis* was effective against many pests (Martin and Bonneau, 2006; Cranshaw, 2008; Shaukat *et al*., 2010). Successful results were reported in studies in which many bacterial and biological agents were used against *N. sertifer* and *D. pini,* and these were recommended in the fight against pests (Mohamed *et al*., 1982; Inmaculata *et al*., 2001; Kees and Amanda, 2013; Van Frankenhyzen and Tonon, 2013). *B. thrugiensis* has been applied previously against *D. pini* in the fight in Artvin for trial purposes, and it was emphasized that this could be successful (Aksu, 2010). Plant-based insecticides have been used together with the organic agriculture practices. The most well known among these are azadirachtin, pyrethrum, rotenone, nicotine, ryania, sabadilla, quassine and plant oils (Güncan and Durmuşoğlu, 2004).

This study was designed to investigate the impact of pyrethrum and *B. thuringiensis* biopesticides on *N. sertifer* and *D. pini* pests and to assess the applicability for future control of these pests in the country.

MATERIALS AND METHODS

This study was conducted in the Scotch pine forest areas of Artvin Regional Forestry Directorate and Borçka Forestry Operation Directorate during 2016-2017. Both *N. sertifer* and *D. pini* larvae were collected from fresh Scotch pine shoots using hand pump and placed in tulle cages, before application of biopesticides. Different doses of biopesticides were applied against the larvae during *in vitro* conditions (Table I).

The 2nd and 3rd instar larvae of *D. pini* were collected in the 1st week of May, and the 2nd and 3rd instar larvae of *N. sertifer* were collected together with the branches of the young Scotch pine trees from which they fed on in the 2nd week of April. These samples were then brought to the Forest Entomology Laboratory in Forest Engineering Department of the Faculty of Forest at Artvin Çoruh University. The larvae that were brought with branches of the trees were placed in wire mesh cages with a size of 20x20x30cm as 20 larvae in each cage. The Scotch pine branches were submerged in moist flower turf to ensure that they remained humid. Biopesticides were applied at a pre-defined dosage (Table I) and monitored at every 12 hours to count and note the dead controls. The numerical data obtained in this process were evaluated with SPSS 15.0 package program. For the purpose of determining the effect of the biopesticides and their dosages on the death of *N. sertifer* and *D. pini* larvae, the One-Way variance analysis (ANOVA) was applied with numerical data. Using the Duncan Multiple Comparison Test we determined the most effective pesticide(s).

Table I.- Sampling organization.

Trade name of the pesticide	Dose	No of cages	No of larvae/ cages
Pyrethrum	150ml/100 lt	10	20
Spruzit Neu	300ml/100 lt	10	20
	600ml/100 lt	10	20
Bacillus	100g/100 lt	10	20
thuringiensis	300g/100 lt	10	20
Dipel DF	500g/100 lt	10	20

RESULTS

Assessing the impact of pesticides on pests revealed that all dosages applied in this study carried an inhibitory effect but at various levels. However, we noticed differences among six different slides applied to *N. sertifer* and *D. pini* larvae (Table II).

Table II.- One-way ANOVA results showing the effects of the pesticides and the doses applied on the larvae of *N. sertifer and D. pini.*

Period of development	Degree of freedom (df)	F value	Significance level (p)
N. sertifer larvae	5	72.38	0.001
D. pini larvae	5	70.12	0.001

Impact of biopesticide applications against the *D. pini* and *N. sertifer* larvae showed that pyrethrum had the same effect with as low as 150 ml/100 dose and as high as 300 ml/100 l dose. In the trial experiment, the efficiency of Dipel at 100 g/100 dose and pyrethrum at 600 ml/100 l dose showed comparable results. In laboratory experiment, all doses of Dipel, which had *B. thuringiensis*, showed the similar effects. The most effective death rates needed for the larvae of both pests to which biopesticide was applied was *B. thuringiensis* (Dipel DF 100 g/100 l), Dipel DF 300 g/100 l, Dipel DF 500 g/100 l dose applications (Table III).

The insecticidal effect of the biopesticides was assessed against *D. pini* larvae. It was found that pyrethrum (Spruzit Neu) was effective at 150 ml /100 l (59.5%); at 300 ml/100 l (62%); at 600 ml/100 l (78.5%) while Dipel DF was effective at 100 g/100 l (85.5%); at 300 g/100 l (93.5%); at 500 g/100 l (95.5%), respectively (Fig. 1).

The insectisidal effect of biopesticides for *N. sertifer* larvae was pyrethrum (Spruzit Neu) at 150 ml/100 l (55.5%) and followed by at 300 ml/100 l (66%), at 600 ml/100 l dose, at a (85.5%). Dipel DF was effected at 100 g/100 l (75%); at 300 g/100 l (86.5%); at 500 g/100 l (95%) (Fig. 2). The highest larva mortality was observed on the 4th and 6th days after the pesticide application in both species.

Table III.- Effects of the pesticides and the doses applied on the larvae and adults of *N. sertifer* and *D. pini* (Duncan Multiple Comparison Test p=0.05). Values are Mean±SD.

Trade name of the pesticide	Dose	*N. sertifer*	*D. pini*
Pyrethrum (Spruzit Neu)	150ml/100 lt	11.9±1.0 c	11.1±1.0 d
	300ml/100 lt	12.4±1.1 c	13.2±1.3 c
	600ml/100 lt	15.7±1.3 b	17.1±0.8 b
Bacillus thuringiensis (Dipel DF)	100g/100 lt	17.1±0.9 b,a	15.0±1.1 c
	300g/100 lt	18.7±0.7 a	17.3±0.9 b
	500g/100 lt	19.1±0.6 a	19.0±0.8 a

DISCUSSION

Previous investigations have highlight that different biopesticides have varied intensities against *N. sertifer* in *in-vitro*. While the lethal effect of *B. thuringiensis* against *N. sertifer* larvae was found with 75-95% in the current study. This rate has been proposed as 35-71% by Inmaculata *et al*. (2001) and as 20.7% by Van Frankeyhzen (2009). While the lethal effect of *B. thuringiensis* against

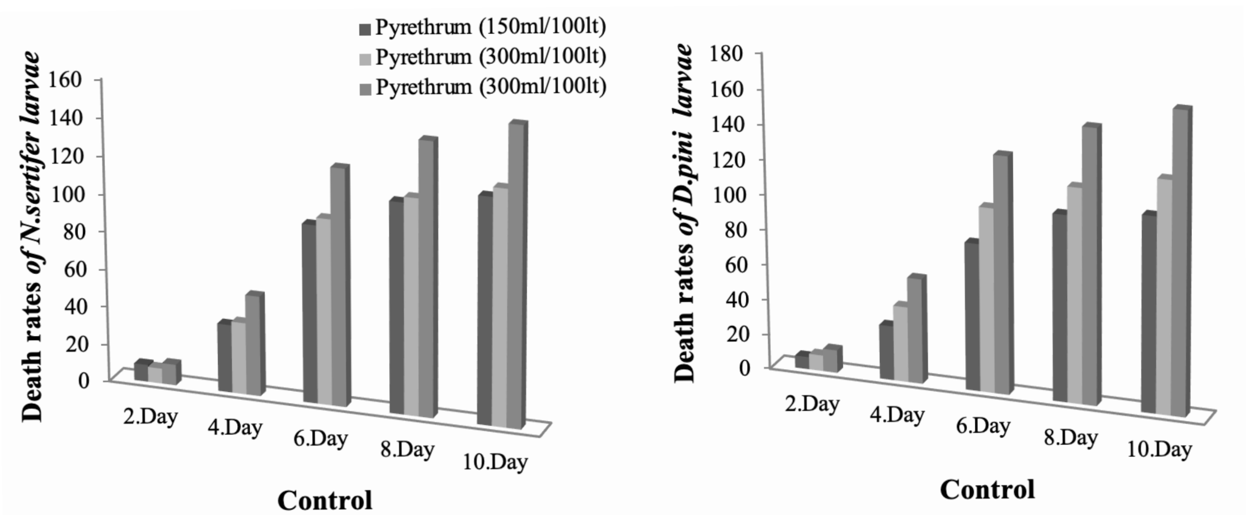

Fig. 1. The effectiveness of different doses of Pyrethrum on the larvae of *N. sertifer* and *D. pini*.

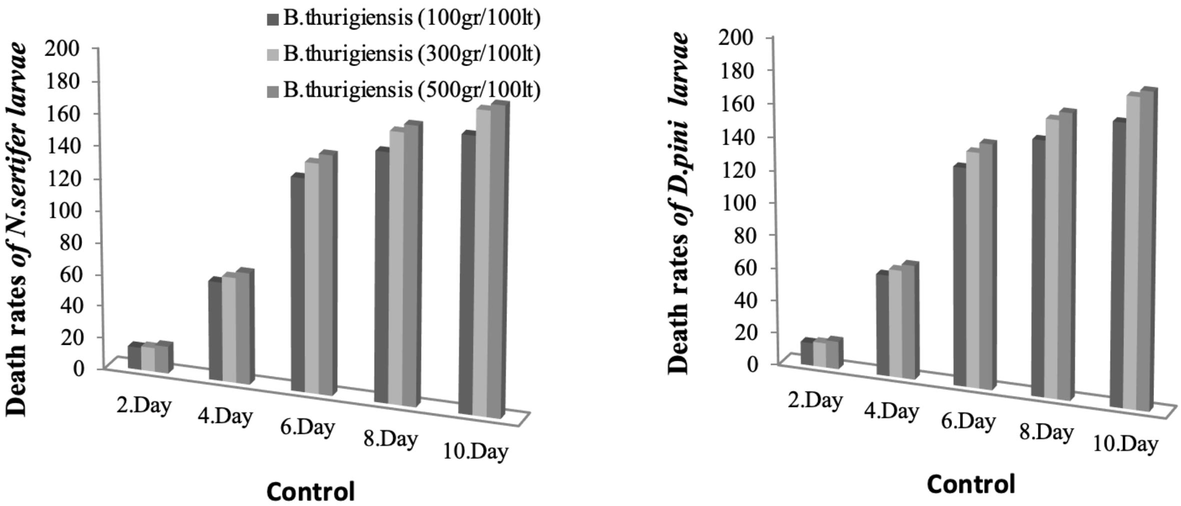

Fig. 2. The effectiveness of different doses of Dipel ® DF BT on the larvae of *N. sertifer* and *D. pini*.

D. pini larvae was found to be 85.5-95.5%; this rate was determined as 40% by Porcar *et al*. (2008); as 34-80% by Dadaşoğlu *et al*. (2016) and as 15-80% by Van Frankeyhzen and Tonon (2013). These differences could depend upon the genetic diversity of pests or biopesticides compositions. Nevertheless, all previous studies have proven the effectiveness of *B. thuringiensis* against these two pests and some different pests (Göktürk *et al*., 2018).

There is paucity of information indicating the lethal effects of plant-based insecticides on bugs and so far no studies have been conducted on the larvae of *N. sertifer* and *D. pini*. The lethal effect of pyrethrum was determined as 55.5-78.5%; the effect of pyrethrum on *Pristiphora abietina* (Christ, 1791) (Hymenoptera: Tenthredinidae) larvae has been investigated in a study conducted by Göktürk (2017) where it was determined as 71.7-98.8%. In present study, we observed that the dose of the pesticide is directly proportional to the death rates in larvae, a trend which has been be proposed earlier (Van Frankenhyzen and Gringorten, 1991).

It is now inevitable that harmful pests cause epidemic periodically in forest and agriculture areas leading to economic losses in crops. Although they do not cause direct deaths of trees, the damage caused by the *N. sertifer* and *D. pini* generally predispose trees to biotic and abiotic harmful factors. It is therefore imperative to design effective control strategies to safeguard the forests and secure the climate changes. The proposed study investigates the pyrethrum (Spruzit® Neu) and *B. thuringiensis* (DiPel® DF) biopesticides against *N. sertifer* and *D. pini* larvae in laboratory conditions. Results their effectiveness against both tested pests even at low doses, which underline the economics and affordability of the farmers. It was also observed that the death rates increased especially on the 4th and 6th days post-pesticide applications highlighting the extent of responsiveness.

CONCLUSION

Based on these finding, it can be concluded that Pyrethrum and *B. thuringiensis* biopesticides should be applied against *N. sertifer* and *D. pini* larvae in the field conditions. In case successful results are achieved in *in-vivo* conditions, it will have great importance to transfer them to use in the fight against pests.

Statement of conflict of interest

Authors have declared no conflict of interest.

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