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Effects of Some Plant Extracts and Bensultap on *Trichoferus griseus* (Fabricius, 1792) (Coleoptera: Cerambycidae)

[1]*Hasan Sungur Civelek and* [2]*Aysen Melda Çolak*

[1]Department of Biology, Faculty of Science and Art, Mugla University, Kötekli, Mugla, Turkey
[2]Sivasli Vocational School, Usak University, Sivali, Usak, Turkey

Abstract: Aqueous extracts prepared from seven plant species, *Cyclamen mirabile* Hildebr., 1906 (Pirumilaceae), *Fritillaria bithynica* Baker, 1874 (Liliaceae), *Juglans regia* L., 1753 (Juglandaceae), *Muscari neglectum* Guss., 1842 (Liliaceae), *Nicotiana tabacum* L., 1753 (Solaneceae), *Sternbergia candida* Mathew and Baytop, 1979 (Amaryllidaceae), *Urginea maritima* L., 1872 (Liliaceae) were tested for their insecticidal activity against *Trichoferus griseus* F. (Coleoptera: Cerambycidae) on *Pinus brutia* L. in the laboratory in 2007. Insecticidal effects of aqueous plant extracts were compared to the application of an insecticide (Bensultap, Bancol 50 WP) and synthetic chemical preservation (chromated copper arsenate, CCA, Tanalith C), which is widely used as a positive control. The effects of those materials on larvae were also analyzed. The bolts have been impregnated with seven aqueous plant extracts and Bensultap and CCA by using a vacuum desicator. Then, larvae of *T. griseus* were placed on the treated and non-treated bolts. Bensultap was the most effective and more significant in reducing the number of larvae in all the trials when compared to the other treatments. The effects of six plant extracts on the larvae were statistically similar to each other except for *U. maritima*. The effects of Tanalith C and *U.maritima* were statistically similar to control. Bensultap would be a welcome addition for the control of wood pests. Aqueous extracts of the six plant species exhibited insecticidal activity are potential candidates as new organic insecticides.

Key words: *Trichoferus griseus* · *Pinus brutia* · Insecticidal activity · Plant extracts · Bensultap · Tanalith C

INTRODUCTION

The fact that wooden buildings that have been standing for hundred of years shows that wood can be used for years if treated properly. These advantages make wood more attractive for construction and furniture production. However, being an organic substance, wood has limited natural endurance. One of the most important pests on wood is *Trichoferus griseus* (Fabricius, 1792) (Coleoptera: Cerambycidae). Larvae cause damage: larvae primarily mine into the wood and adult lay eggs into scars on the surface of wood [1-3]. Wood can be effectively protected from borer infestation by impregnating the wood with pesticide effective chemicals. Impregnating wood by applying suitable chemicals is the most preferred preservation method but some toxic chemicals, used for wood preservation, contain serious pollution risks to the environment [4,5]. There are many effective preservative chemicals used against wood pests in the world. CCA (chrome, copper, arsenic) formulations are the best known brand name in the world. These products are widely accepted by authorities around the world for permanent and long term protection against fungal decays, soft rot, insect groups, termites and borers. Tanaliths are widely used in pressure applications with outstanding results [6].

Consumers have become concerned about pesticide residuals on food crops and governments have responded by legislating and restricting the usage of synthetic pesticides. Efficacious botanical derivatives can provide an alternative to synthetic pesticides and agrochemical companies have started to focus on these alternatives [7].

Recently, herbal extracts that are not harmful to the environment, have been shown to be effective natural preservatives [8-10]. Grace and Yamamoto [11] compared the resistance of the samples from core wood of Alaska cedar, redwood and teak species with the wood processed

Corresponding Author: Hasan Sungur Civelek, Department of Biology, Faculty of Science and Art, Mugla University, Kötekli, Mugla, Turkey

with chromated copper arsenate (CCA). They stated that the extracts from core wood performed better than CCA. Woods and Cookson [6] reported the effects of chlorothalonil (2,4,5,6-tetrachloro-isophthalotrile) against marine borers, *Limnoria* spp. (Crustaceae). Chlorothalonil is a known fungicide. They have found that wood can be effectively protected from marine borer infestation by impregnating the wood with a pesticidally effective amount of chlorothalonil. However, chlorothalonil is a function of not only the specific type of wood to be protected, but also of the anticipated environment in which it is used. Nakayama *et al*. [12] have investigated the effects of corrosion resistant features of rubber and resin extracts against termite on wood. It was reported that Guayule is not only resistant to pest and microorganisms but is also a secure, renewlable and alternative tree preservative. Sen *et al*. [5] reported that they have used the extracts of *Pinus bruti* L. barks, valex (the valonia extract of *Quercus ithaburensis*), gallnut powders (*Q. infectoria* Oliv.) and sumac leaves (*Cotinus coggyria* Scop.) against *Rhagium inquisitor* (L.) (Coleoptera: Cerambycidae) larvae. They compared the effects of these extracts with those of chromated copper arsenate (CCA). Their studies showed that the development of larvae in the treated wood has been prevented due to extracts and the larvae could not live on the woods treated by the plant extracts of more than 4% of concentrations.

Wahab *et al*. [13] proved after resistance tests that bamboo e xposed to petrol (oil) healing process has lower performance but pretty higher resistance to the biologically- destroying factors. Li and Zhang [14] investigated the oviposition behaviour and response of adult females of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) to short bolts of *Pinus massoniana* Lamb. trunks treated with hexane extracts of larval frass of *M. alternatus*. The results suggest that the preference of a possible oviposition is deterrent in hexane extracts of larval frass of *M. alternatus*.

Here we are reporting the insecticidal effects of aqueous plant extracts of the *Sternbergia candida* Mathew and Baytop, 1979 (Amaryllidaceae), *Cyclamen mirabile* Hildebr., 1906 (Pirumilaceae), *Muscari neglectum* Guss., 1842 (Liliaceae), *Fritillaria bithynica* Baker, 1874 (Liliaceae), *Urginea maritima* L., 1872 (Liliaceae), *Juglans regia* L., 1753 (Juglandaceae), *Nicotiana tabacum* L., 1753 (Solaneceae) against *T. griseus* on *Pinus brutia* L.

MATERIALS AND METHODS

Source plants: Plants were collected from wild areas in Mugla province, western Turkey in 2007 during the months of May through June and brought to laboratory for extraction. Voucher specimens of each plant were deposited in the Entomology Laboratory, Biology Department of Faculty of Science and Art, Mugla University, Turkey.

Extract preparation: Plants were washed thoroughly to remove any soil debris. Those not immediately used were stored in paper bags at 8 °C until needed. The plants were initially cut into pieces and then placed in a blender to break them into very small pieces. Each plant sample (2 g) was extracted with 100 ml water using a Soxhlet hot water extractor (PILZ, Heraus-Witmann, Heidelberg, Germany) for 4 h. Extracts were stored in a sealed bottle at 5°C until use [15].

Insect source: An experimental colony of *T. griseus* was formed from insects collected from wood depots, wooden homes and some furnitures in Mugla province. According to gender, newly emerged adults were reared individually in iron-screened cages (75 x 65 x 50 cm) on 1-2 year old of *P. brutia* twigs at 25°C and under a 12L:12D photoperiod. Two weeks later, females and males were paired. The females oviposited in 15-18 days after emergence. We selected 18-25 days old females for the oviposition and the females were allowed to oviposit [14]. The first star larvae of 7 days old were used in the tests.

Impregnating studies: The trunks of healthy specimens of *P. brutia* were cut into measuring 20 ×15 ×1.5 cm without nodes as 25 bolts for each trial. The cut ends of the pine bolts were then sealed with liquid paraffin (melting point: 56-58 ºC) and stored in black plastic bags at room temperature (65% ±5 moisture at 20±2ºC) until used in the tests. All natural and synthetic chemicals have been impregnated into the wood bolts by using a vacuum desicator.

Each plant extract was diluted to 1:25 v/v in distilled water immediately before use (4). Also insecticide bensultap (Bancol 50 WP, Takeda Chemical Industries) and synthetic chemical preservation (CCA, Tanalith C) were used with recommended dosages (recommending dosages are: %0.01 for bensultap, %6 for Tanalith C).

However, untreated bolts without any chemical applications served as a negative control for each trial.

Before the vacuum processes, samples were weighed. Bolts were treated by drawing a vacuum (-90 kPa) for 30 minutes, introducing the preservative under vacuum and the immediately releasing the vacuum. The bolts were left to absorb preservatives for 30 minutes at 100 mm Hg pressure in a vacuum desicator. After treatment, all bolts (except untreated controls) were wrapped in aluminum foil and stored for two weeks at room temperature to ensure fixation of preservatives. Bolts were then unwrapped and left to air-dry for two weeks [6].

Larva test: The 25 impregnating bolts for each trial with seven plant extracts, one insecticide (bensultap), one synthetic chemical preservative substance (Tanalith C) and untreated bolts were used in this study. The larvae holding in the cages were taken and placed into the bolts infested with larvae and then placed in an incubator at 23.5 ºC, 65% moisture under 12L:12D photoperiod. But first of all, some gallery paths were opened by a drill on the surface of bolts to ease *T. griseus* larvae's feeding.

The experiment was conducted with 25 replicates each. One larva was placed into each experimental bolt. Observations were carried out to state naturals that are alternative to synthetic preservation substances and the number of live larvae were counted and recorded. Each trial was terminated after one year because the larvae had reached adulthood.

Data analysis: Data were analyzed using one- and two-way analysis of variance (ANOVA) and means were separated using Tukey's HSD quantile function, using SPSS 11.0. All tests were conducted at á = 0.05 level.

RESULTS

Bolts were weighed before and after the injection of preservative. The results are shown in Table 1.

Each trial consisted of measuring the number of live larva in 25 bolts over a period of 12 months. Results of the trials for all applications are shown in Table 2.

Results show that bensultap was the most effective treatment since it significantly reduced the number of larva in comparison to the other treatments ($p< 0.05$). Plant extract treatments except for *U. maritima* also reduced the number of larva compared to Tanalith C and uncontrol treatments ($P<0.05$). The effects of the other 6 plant extracts on larvae were similar to each other and no statistically significant difference has been observed ($p> 0.05$). We expected the lowest effect at control group but *U.maritima* and a synthetic preservative Tanalith C were added and no difference was seen statically ($p> 0.05$). The most significant finding is that these 6 plant extracts were more effective than Tanalith C, a chemical that is used in sector and harmful to the environment and health.

Where natural and synthetic chemicals were applied as preservatives on *T.griseus* larvae in incubators in laboratory; bensultap had the highest effect but plant

Table 1: The weights of samples before and after preservation

Bolt No	Exctract	First weight (gr)	Weight after preservation
1	*Nicotiana tabacum*	97.17	112.55
2	*Nicotiana tabacum*	94.07	112.19
1	*Sternbergia candida*	84.42	97.2
2	*Sternbergia candida*	77.65	137.05
1	*Cyclamen mirable*	98.68	126.07
2	*Cyclamen mirable*	102.08	120.48
1	*Muscari neglectum*	70.79	80.84
2	*Muscari neglectum*	102.9	117.01
1	*Juglans regia*	85.58	94.74
2	*Juglans regia*	85.49	99.91
1	Bensultap	100.35	135.98
2	Bensultap	85.46	119.43
1	*Fritillaria bithynica*	80.94	118.58
2	*Fritillaria bithynica*	80.81	141.34
1	*Urginea maritima*	90.5	97.51
2	*Urginea maritima*	82.81	131.89
1	Tanalith C	75.11	154.63
2	Tanalith C	76.10	167.41
1	Untreated	100.01	-
2	Untreated	100.03	-

Table 2: The table shows the effects of seven plant extracts, bensultap and Tanalith C on *Trichoferus griseus* larvae in *Pinus brutia* bolts. Table indicates the average number of live (±SE) larvae from first day until the end of trial

Extracted plant	Number of alive larvae
Bensultap	0,000±0,00 a
Cylamen mirable	0,5714±0,05 ab
Fritillaria bithynica	0,2222±0,04 ab
Juglans regia	0,4000±0,05 ab
Muscari neglectum	0,4286±0,05 ab
Nicotiana tabacum	0,1818±0,04 ab
Sternbergia candida	0,5000±0,05 ab
Tanalith C	0,6667±0,05 b
Urginea maritima	0,6667±0,05 b
Control	0,6667±0,05 b

Different letters within a column indicate differences of $P < 0.05$

extracts (*Nicotiana tabacum*, *Fritillaria bithynica*, *Muscari neglectum*, *Juglans regia*, *Sternbergia candida*, *Cylamen mirable*) had higher effects on larvae than Tanalith C.

DISCUSSION

T. griseus has been a major wood-destroying pest in Turkey in the recent years. Producers and owners have been using some synthetic wood preservatives, especially Tanalith C, against wood pests for a long time. Unfortunately, while these heavymetal preservatives provide substantially improved protection against borers, they can also significantly reduce strength of wood, leaving the piles brittle. In addition, these materials raise significant environmental concerns because of the highly toxic nature of the heavy metals they contain [6]. Also, non-judicious use of those chemicals raises a serious concern about the possibility of resistance developing in wood pest larvae. We used plants extracts and bensultap and Tanalith C as an insecticide against pests: Tanalith C was used as a positive control because it is widely used for control of wood pests.

Improving natural preservatives instead of chemicals is necessary. Some hopeful results were given nearly from all studies where natural plant extracts were used as an alternative to the synthetic preservatives. These plants are abundant in Turkey and could serve as an inexpensive and organic alternative to commercial insecticides. Research is in progress with six potential insecticides to identify the active ingredients in the insecticidal activity of these plants.

There is relatively little information about insecticidal activities of *U. maritima.* The active compounds are L-azetidine-2-carboxylic acid (=AZA) and the bufadienolides: scilliroside, scilla glycoside and aglycones [16]. Hassid *et al*. [17] were the first to examine the insecticidal properties of extracts of *U. maritima.* They showed that the foliage of *U. maritima* was highly toxic to lepidopterus larvae. Methanol extracts of dried leaves (which contained AZA), when incorporated into artificial diet, also caused 100% mortality. Civelek and Weintraub [15] applied aqueous extracts of *U. maritima* against *Liriomyza trifolii* (Diptera: Agromyzidae). There was no significant difference between the numbers of live larvae found 1:25 dilution of *U. maritima* treated to leaves versus cyromazin-treated leaves in all trials. We used 1:25 dilution of all extracts as stated by Civelek and Weintraub [15]. But *U.maritima* was found ineffective on *T. griseus* larvae in our study. We thought that while the extract of *U.maritima* was absorbed by the living cells of plant tissues in tomatoes leaves, it is possible that the extract could not absorbed by the dead cells of the wood tissues.

In search for additional active ingredients to control wood pests, an old group of insecticides and wood protecting chemicals, based on secretions of marine annelids (*Lumbricus* spp.) are being examined for new applications. These synthetic insecticides are derivatives of nereistoxin and include cartap, bensultap and thiocyclam. The mode of action of these insecticides is as an antagonist, blocking cholinergic transmission resulting in paralysis and insect death. Once eaten by the insect, these insecticides are metabolically converted to nereistoxin, which interacts with nicotinic acetycholine receptors [18]. There have been few trials with synthetic nereistoxin insecticides for the control of pests [8]. Bensultap has primarily been used for the control of coleopterrous pests [19]. But there are no studies on wood pests. Civelek and Weintraub [20] applied bensultap against *L. trifolii*. There was no significant difference between the numbers of live larvae versus cyromazin-treated leaves in all trials.

This study shows that bensultap is the most effective in controlling the larvae of *T. griseus*. Bensultap would be a welcome addition for the control of wood pests. Aqueous extracts from these six plant extracts exhibited activity and are potential candidates as new organic insecticides.

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