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Insecticidal activity of three plant extracts against adult *Ips typograpgus* L. under laboratory conditions

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Three plant extracts - *Origanum vulgare* subsp. *hirtum* essential oil (EO), *Monarda fistulosa* EO, and a hexane fraction of *Tanacetum cinerariifolium*, were evaluated and compared regarding their insecticide activity under laboratory conditions against the adults of the European spruce bark beetle, *Ips typographus*. The viability of *I. typographus* was affected with all tested plant extracts. The insecticidal effect varied among the extracts and concentrations used. Generally the mortality of beetles increased with the concentration of extracts and a significant positive correlation between the cumulative mortality and the extract concentration was confirmed by Pearson's correlation analysis ($r = 0.910$, $p = 0.012$ for *O. vulgare* subsp. *hirtum*, $r = 0.937$, $p = 0.005$ for *T. cinerariifolium*, $r = 0.814$, $p = 0.048$ for *M. fistulosa*). At the highest extract concentration (10%), the total cumulative mortalities reached 98% for *O. vulgare* subsp. *hirtum* and *T. cinerariifolium* extracts at the end of the bioassay (four days after treatment). The exposure of beetles to *M. fistulosa* extract decreased the survivability of *I. typographus* the most (15%) and was followed by *T. cinerariifolium* (38%) and *O. vulgare* subsp. *hirtum* (43%). Log-rank test showed no significant difference in the survival probabilities among the extracts. These are the first tests on the insecticidal effect of plant extracts from *Tanacetum cineranifolium* and *Monarda fistulosa* against the European spruce bark beetle *Ips typographus*.

Key words: plant extracts, *Origanum*, *Monarda, Tanacetum*, efficacy, spruce bark beetle

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INTRODUCTION

Forests are immensely valuable natural resources and their conservation and sustainable development is the basis for their long-term use. As part of forest ecosystems, bark beetles (Coleoptera, Curculionidae: Scolytinae) are one of the most deleterious insect pests in coniferous forests throughout Europe. They usually attack recently dried or physiologically weakened trees, but during mass attacks, they can even damage healthy standing trees, resulting in huge economic loss (Takov et al. 2012). The European spruce bark beetle, *Ips typographus* (Linnaeus, 1758) stands out as the most economically significant pest of spruce forests and subsequent monitoring has been carried out in Central Europe as a result of its calamities (e.g. Germany, Austria, Poland, Slovakia, Czech Republic and Bulgaria) (Rossnev et al. 2005; Grodzki et al. 2019; Kunca et al. 2019; Lubojacký et al. 2019). The Norway spruce *Picea abies* (L.) H. Karst. the principal host tree of *I. typographus*, and it is one of the most commercially important coniferous species in Europe (Bentzet et al. 2019). In healthy forest ecosystems, *I. typographus* attacks weakened or dying trees and plays an essential role in the natural process of wood decomposition (Müller et al. 2008). However, extreme weather events such as extensive drought, storms, or wildfires can cause outbreaks of this pest. Spruce bark beetle infestations can affect enormous areas with thousands of trees. Until now, the most commonly used control method against *I. typographus* has been sanitary measures, which include removing infested trees (Wermelinger 2004).

In the search for alternatives to conventional insecticides, essential oils extracted from aromatic plants have been widely investigated. Their toxicity in pests, as well as their inhibitory and repellent effects on insects have been of particular interest in the last decade (Garrido-Miranda et al. 2022). Plant-produced defense compounds, formerly assumed to be merely bioproducts (secondary metabolites) of primary metabolism, are extraordinarily diversified in their structure due to millennia of herbivore selection pressure (Mithöfer & Boland 2012). Therefore, these plant metabolites are now referred to as specialized metabolites rather than secondary ones (Pichersky & Lewinsohn 2011). Examples of extracts used long ago include pyrethrum found in Asteraceae, capsaicin from chili pepper extract, and azadirachtin isolated from neem seeds (Pavela 2007; Benelli et al. 2017). The use of natural products as an alternative to synthetic insecticides is a priority of modern crop production. Four main types of botanical products are currently used for insect control, namely rotenone, neem, pyrethrum, and essential oils (Isman 2006). In recent years, essential oils (EOs) have been one of the most popular alternative methods of arthropod control due to their wide source of bioactive constituents (Regnault-Roger et al. 2012; de Oliveira et al. 2014). They are complex mixtures containing from 20 to 60 components in various concentrations, with two or three main components usually constituting 20–70% of their composition (Bakkali et al. 2008).

Each plant species produces EOs with a specific mixture of chemical constituents (War et al. 2012; Zaker 2016). EOs may have toxic properties against insect pests or may interfere with insect oviposition, growth and reproduction. Essential oils from many plant species have been investigated as an alternative to synthetic insecticides (Isman 2000; Pascual-Villalobos & Ballesta-Acosta 2003; García et al. 2005). The insecticidal constituents of many plant EOs are mainly monoterpenoids (Ahn et al. 1998; Kim et al. 2003), and due to their high volatility, they often have strong fumigant effects with insecticidal and acaricidal activity (Ho et al. 1997; Chang & Ahn 2002; Szczepanik et al. 2018).

Oregano, *Origanum vulgare* Linnaeus, 1753 (Lamiales: Lamiaceae), is an important aromatic plant and a rich source of terpenoid components used in medicine, the food industry, and agriculture. Oregano essential oil (OEO) has been used for a long time against several microorganisms found in stored products, with low animal toxicity and rapid degradation in the environment (Plata-Rueda et al. 2021). The essential oils obtained from Greek oregano, *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart, were found to be rich in carvacrol, thymol, γ-terpinene, and p-cymene. These EOs and their main constituents, carvacrol and thymol, were tested for insecticidal and genotoxic activities on *Drosophila* Fallén, 1823 (Karpouhtsis et al. 1998), and on *Diabrotica virgifera virgifera* LeConte, 1868 (Toshova et al. 2022). Plata-Rueda et al. (2022) reported that OEO exhibits potent insecticidal effects against storage pests. The ovicidal effect of *O. vulgare* EO on silver leaf whitefly *Bemisia tabaci* (Gennadius 1889), common green bottle fly *Lucilia sericata* (Meigen, 1826), Angoumois grain moth *Sitotroga cerealella* (Olivier, 1789), carob moth *Ectomyelois ceratoniae* (Zeller, 1839), and bean weevil *Acanthoscelides obtectus* (Say, 1831) has been established (Valizadeh et al. 2021). OEO demonstrated high toxicity to *Sitophilus oryzae* (Linnaeus, 1763) with LD_{50} values of 0.08–0.11 mg/cm^2 (Abdelgaleil et al. 2016). Gonzalez-Coloma et al. (2013) also established that estragole and (+)-fenchone contained in the OEO were very effective against *S. oryzae*, *Callosobruchus chinensis* (Linnaeus, 1758) and *Lasioderma serricorne* (Fabricius, 1792). The insecticide potential of Greek oregano, *Origanum vulgare hirtum*, EO was evaluated against *Diabrotica virgifera virgifera* (WCR) adults under laboratory conditions (Toshova et al. 2022).

Zhilyakova et al. (2009) reported that *Monarda fistulosa* Linnaeus, 1753 (Lamiaceae) EO exhibited antibacterial activity. Studies on *Monarda punctata* Linnaeus, 1753 and *M. citriodora* Cervantes ex Lagasca y Segura,1816 essential oils (with thymol and carvacrol as dominant compounds) exhibited repellent effects against adult mosquitoes (McCann et al. 2006) and *Drosophila melanogaster* Meigen, 1830 even at low concentration levels (Harrison et al. 2008). *Monarda fistulosa*, the wild bergamot or bee balm, is native to North America, but nowadays is widespread in different regions. It has been cultivated as an essential oil plant since 1637. The above-ground parts contain essential oil, terpenoids, polyphenols (hydroxycinnamic acids, flavones, flavonoids, anthocyanins), monardein, amino acids, bitterness, tannins, cellulose, pectins, vitamin C (Oparin et al. 2000). The leaves contain thymol and carvacrol, hydroxycinnamic acids and flavone glycosides.

Plants of the Asteraceae family have been investigated in several studies as a potential source of biopesticides with insecticidal properties (Umpiérrez et al. 2012; Ikeura et al. 2012; Czerniewicz et al. 2018) and species of the *Tanacetum* genus are the most frequently studied for this purpose. They produce different classes of secondary metabolites, including flavonoids, phenolic acids, sesquiterpene lactone, monoterpene, diterpene, glycosides, alkaloids, phytosterols, heterocyclic compounds, polyacetylenes and others. Many species of this genus are used as medicinal plants. They have shown growth regulating, phytotoxic, antiulcer, anthelmintic, antifungal, antioxidant, insecticidal and antimicrobial characteristics (Bukhari et al. 2007; Kumar & Tyagi 2013). Golden buttons or tansy (*Tanacetum vulgare* Linnaeus, 1753), the most studied *Tanacetum* species, contains EO that showed antifungal, anthelmintic and antibacterial properties (Mikulášová & Vaverková 2009; Stevovic et al. 2009; Bączek et al. 2017; Ivănescu et al. 2018).

The chemical profile of its EO includes thujone, camphor, borneol, α-pinene, 1,8-cineole, bornyl acetate, sabinene, and camphene (Kumar & Tyagi 2013; Mureşan 2015), however, it is unstable and changes depending on the geographic origin of plants (Mockute & Judzentiene 2004; Stević et al. 2009). Extracts from feverfew, *Tanacetum parthenium* (Linnaeus, 1753) Schultz-Bipontinus contain volatile oil, sterols (Rateb et al. 2007), a range of sesquiterpene lactones (Kaplan et al. 2002), flavonoids such as luteolin and apigenin (Wu et al. 2006), various flavone glycosides (Williams et al. 1999) and tannins (Marete et al. 2009). EOs from both *Tanacetum* species exhibit anti-nutritional and repellent effects against adults and larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Lazarević et al. 2021). Insecticidal, antifeedant and growth inhibitory effects were also observed against *Spodoptera littoralis* (Boisduval, 1833) larvae (Pavela et al. 2010). *Tanacetum chiliophyllum* (Fisch. & C.A. Mey. ex DC.) Schultz Bip. 1844 has shown promising insecticidal activity against the grain weevil, *Sitophilus granarius* L. (Polatoğlu et al. 2011). Compounds isolated from *T. chiliophyllum* possess cytotoxic, antimicrobial, and acetylcholinesterase, butyrylcholinesterase inhibitory effects (Polatoğlu et al. 2011). Many sesquiterpene lactones and flavonoids as anti-inflammatory agents have been isolated from *Tanacetum sinaicum* (Fresen.) Delile ex K. Bremer & Humphries 1993 (Hegazy et al. 2015). Pyrethrins, which are produced by Dalmatian (wild) pyrethrum, *Tanacetum cinerariifolium* (Trevir.) Schultz-Bip, 1844 were well known for their insecticidal effects (Kumar et al. 2005).

The aim of the present study was to evaluate and compare the insecticide activity of extracts from three plants species (*O. vulgare hirtum*, *M. fistulosa* and *T. cinerariifolium*) against the adults of the European spruce bark beetle, *I. typographus,* and to establish insect susceptibility to the extracts under controlled laboratory conditions.

MATERIAL AND METHODS

Insects

Adults of *I. typographus* were collected from fallen trees of Norway spruce on Vitosha mountain, near Sofia (Bulgaria) at the beginning of August 2022. The beetles were kept with pieces of bark of *P. abies* in a refrigerator at 4 ± 1°C until treatments were set up, which took no more than 7 days. Before bioassays, live adult beetles were separated from the bark pieces and placed in cohorts of 20 unsexed individuals in glass Petri dishes (diameter: 9 cm).

Plant material and phytochemical analysis

Plant material – aerial parts of *O. vulgare hirtum* and flower heads of *M. fistulosa* and *T. cinerariifolium* were collected from the *ex situ* collection from the Institute of Biodiversity and Ecosystem Research. Essential oils from *O. vulgare hirtum* and *M. fistulosa* were extracted on a Clevenger apparatus by water distillation. Plant material of *T. cinerariifolium* was extracted with hexane by maceration at room temperature for 24 hours. After filtration and evaporation to dryness, the hexane fraction was obtained.

Chemical composition of the essential oils of *O. vulgare hirtum* and *M. fistulosa* and the hexane fraction of *T. cinerariifolium* were analyzed on a Thermo Scientific Focus GC coupled with Thermo Scientific DSQ mass detector operating in the electron ionisation mode at 70 eV. A DB-5MS column (30 m × 0.25 mm × 0.25 μm). The temperature program for essential oil analyses was described by Traykova et al. (2019) and for hexane fraction by Berkov et al. (2021). The components were identified by comparing their mass spectra and retention indices (RI) with those of authentic standards, the National Institute of Standards and Technology (NIST) spectra library, and literature data (Adams 2007).

Insecticidal activity bioassay

The insecticidal effects of plant extracts on *I. typographus* were determined by the filter paper disc method (Mudrončeková et al. 2019). Petri dishes with a disc of filter paper (90 mm diameter; Whatman No. 1) on the bottom were used in the bioassay. The plant extracts were diluted in hexane (Sigma-Aldrich®, purity ≥99%) to obtain the following concentrations – 0.5%, 1%, 2.5%, 5%, 7.5%, and 10%. As many as 100 µl of each solution were evenly pipetted on to the surface of filter paper discs in the Petri dishes. The dishes were left open for 10 minutes to allow solvent evaporation, and the cohorts of 20 bark beetles were deposited in the center of treated paper discs. Control treatments contained paper discs treated with 100 µl of hexane. The Petri dishes with beetles were incubated at 20-22ºC for four days, and mortality was recorded at 24-hour intervals. Individuals were considered dead if no movements of the appendages were observed after a gentle touch of the body with tweezers. Dead beetles were removed and placed in individual Petri dishes. Three replicates of each extract concentration and control treatment were made.

Data analysis

The mean cumulative mortality data from the bioassays were corrected for a natural (control) mortality using Schneider-Orelli's formula (Püntener 1981) and arcsine transformed ($n' = \arcsin\sqrt{n}$) before ANOVA was used to determine differences among efficacy of plant extracts. Lethal concentrations LC_{50} and LC_{90} with associated 95% confidence intervals were estimated using probit analysis (Finney 1971). Data for the median lethal concentrations (LC_{50}) and slopes from the probit analyses were subjected to ANOVA. The post-hoc Tukey's HSD test was used to compare the means if significant differences ($p = 0.05$) were detected. Mean survival times of the bark beetles treated with 2.5% solutions of plant extracts were estimated using Kaplan-Meier survival analysis (Kaplan & Meier 1958). Log-rank test was used to determine whether there is a statistically significant difference between survival probabilities estimated for the extracts. All the analyses were performed using Minitab 17® (© 2013 Minitab Inc.).

RESULTS

Phytochemical analysis

Essential oils of *O. vulgare hirtum* and *M. fistulosa*, as well as a hexane fraction of *T. cinerariifolium* were analyzed for their composition by GC/MS and the compounds identified are present in Table 1. Carvacrol and p-cymene were identified as the main components in the essential oil of *O. vulgare hirtum*. Thymoquinone, p-cymene, thymol and carvacrol were identified as the most abundant components of *M. fistulosa* essential oil. Pyrethrins (33.25%) were the main bioactive compounds in the chemical profile of the hexane fraction of *T. cinerariifolium.*

Table 1. Chemical composition of plant extracts included in the insecticide activity bioassay against the adults of *Ips typographus*.

Rt**	Component	Content of components (%)*		
		Origanum vulgare hirtum	*Monarda fistulosa*	*Tanacetum cinerariifolium*
6.99	α-Thujene	0.52	4.72	
7.17	α-Pinene	3.19	5.02	
7.49	Camphene	1.06		
8.11	β-Pinene	0.86		
8.28	Chrysanthemic acid			4.42
8.38	β-Myrcene	1.78		
8.81	α-Phellandrene	0.31		
9.14	α-Terpinene	2.78	0.35	
9.68	p-Cimene	15.61	21.82	
10.41	γ-Terpinene	3.91		
11.03	p-Mentha-1,4(8)-diene	0.50		
11.4	β-Linalool	0.39		
13.58	Terpinen-4-ol	0.98		
15.21	Carvacrol methyl ether	2.29	1.98	
16.02	Thymoquinone		25.41	
17.24	Thymol		19.75	
17.53	Pentanoic acid (Valeric acid)		0.39	
17.57	Carvacrol	48.75	12.24	
18.38	Undecanoic acid			4.47
20.22	Caryophyllene	3.52	0.69	
21.56	1,*E*-11,*Z*-13-Octadecatriene		9.68	
22.6	Cinerin I			8.67
23.94	Jasmolin I			6.62
24.44	Pyrethrin I			1.97
24.59	Caryophyllene oxide	1.32		
25.2	Humulene-1,2-epoxide	0.23		
27.48	Unidentified alkane 1			3.13
28.31	Cinerin II			7.87
29.49	Jasmolin II			5.80
29.57	Pyrethrin II			2.32
30.56	Unidentified alkane 2			1.76
33.5	Unidentified alkane 3			5.05
37.84	(+)-Sesamin			5.09
46.09	Cyclohexyl benzoate			0.28
	Total detected (%)	88.00	91.98	67.52

*values represent % area of components of the total area of chromatogram; blank space – the component not identified for a particular extract; **Rt – retention time obtained by gas chromatogram

Insecticidal activity

Mean mortality in the control treatments ranged from 13.33% to 16.67%. The viability of *I. typographus* was affected with all tested plant extracts. The insecticidal effect varied among the extracts and concentrations used. Generally, the mortality of beetles increased with the concentration of extracts (Fig. 1) and a significant positive correlation between the cumulative mortality and the extract concentration was confirmed by Pearson's correlation analysis (r = 0.910, p = 0.012 for *O. vulgare hirtum*, r = 0.937, p = 0.005 for *T. cinerariifolium*, r = 0.814, p = 0.048 for *M. fistulosa*). At the highest extract concentration (10%), the total cumulative mortalities reached 98% for *O. vulgare hirtum* and *T. cinerariifolium* extracts at the end of the assay (four days after treatment). *Monarda fistulosa* extract killed 100% of beetles as early as two days after treatment with 10%-extract and four days after treatments with concentrations of extract ≥5.0%. The differences in insecticidal activity among the tested extracts depended on the concentration. While *M. fistulosa* extract was the least effective at concentrations ≤1%, it was the most toxic at concentrations ≥2.5%. *T. cinerariifolium* extract was the most effective at concentrations ≤1%, but the least toxic at concentrations ≥2.5%. Significant differences in cumulative mortality recorded four days after treatment among the extracts were observed only for concentrations of 1% ($F_{(2,6)}$ = 38.47, p< 0.001) and 5% ($F_{(2,6)}$ = 10.70, p = 0.010).

A probit analysis was performed to estimate lethal concentrations for plant extracts at a 4-day exposure time (Fig. 1). Among the analyzed extracts, the insecticide effect increased in this order: *T. cinerariifolium* <*O. vulgare hirtum* < *M.fistulosa* (Table 2). The highest toxicity was observed for *M. fistulosa* extract (LC_{50} = 1.93±0.13%, LC_{90} = 2.70±0.15%), however, the effect was not significantly (p > 0.05) different from the two other extracts. The survival analysis was applied to mortality data for a 2.5% concentration of extracts because this concentration was closest to the values of median lethal concentration estimated by probit analysis (Table 2). The probability of *I. typographus* to survive after exposure to extracts decreased with time (Fig. 2) and varied among tested plant extracts (Table 3). The exposure of beetles to *M. fistulosa* extract decreased the probability to survive the most (15%) and was followed by *T. cinerariifolium* (38%) and *O. vulgare hirtum* (43%). Log-rank test showed no significant difference in the survival probabilities among the extracts (Table 4). Mean survival times estimated by Kaplan–Meier survival analysis for *I. typographus* adults treated with 2.5% solutions of plant extracts ranged from 2.58 to 2.80 days (Table 3, Fig. 3).

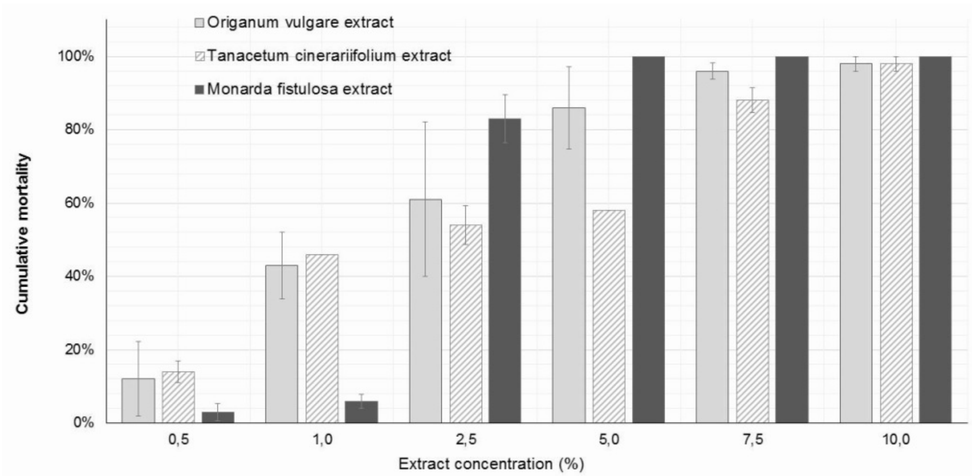

Fig. 1. Mean cumulative mortality* of *Ips typographus* adults four days after treatments with different concentrations of three plant extracts in laboratory bioassays. The error bars represent the standard errors of means. (*corrected for a natural mortality in the control)

Table 2. Results of probit analyses testing insecticide effect of plant extracts against adults of *Ips typographus* in laboratory bioassays.

Plant extracts	**Probit analysis parameters**					
	Lethal concentrations (%) *		**95% fiducial CI (%)**	**Slope ***	***p* ****	**χ^2*****
O. vulgare hirtum	LC_{50}	2.30±0.28	1.72–2.84	0.35±0.04 ab	<0.001	7.58
	LC_{90}	5.94±0.45	5.18–7.01			
T. cinerariifolium	LC_{50}	3.09±0.34	2.38–3.75	0.26±0.03 a	<0.001	3.25
	LC_{90}	8.02±0.61	7.01–9.49			
M. fistulosa	LC_{50}	1.93±0.13	1.59–2.16	1.67±0.33 b	<0.001	1.16
	LC_{90}	2.70±0.15	2.46–3.10			
ANOVA ****	$F_{(2,6)} = 0.18$, $p = 0.44$			$F_{(2,6)} = 11.10$, $p = 0.009$		

*Mean values ± standard errors of means; mean values followed by the same letter are not significantly different (Tukey's HSD test, $p = 0.05$),***p*-value of a slope from regression analysis,***Pearson χ^2 goodness-of-fit test on the probit model ($p = 0.05$, $df = 4$),****ANOVA statistics testing for differences in the mean LC_{50}s and slopes among the extracts. Mean mortality in the controls reached 16.67±3.33% in bioassays with *O. vulgare* subsp. *hirtum* and *T. cinerariifolium* extracts and 13.33±3.33% in bioassays with *M. fistulosa* extract.

Table 3. Results of Kaplan–Meier survival analysis for *Ips typographus* adults treated with 2.5% solutions of plant extracts.

Plant extracts	Mean survival time ±SE (days)	Survival probability estimated for day 4 ±SE	95% confidential intervals
O. vulgare hirtum	2.77±0.16	0.43±0.06	0.38–0.49
T. cinerariifolium	2.80±0.17	0.38±0.06	0.34–0.43
M. fistulosa	2.58±0.12	0.15±0.05	0.14–0.16

Table 4. Results of Log-rank test to determine whether there is a statistically significant difference between survival probabilities estimated by Kaplan–Meier survival analysis for *Ips typographus* adults treated with 2.5% solutions of plant extracts.

Plant extracts	Log-rank test parameters
O. vulgare hirtum × *T. cinerariifolium*	χ^2= 0.114, p = 0.735
O. vulgare hirtum × *M. fistulosa*	χ^2= 3.348, p = 0.067
T. cinerariifolium × *M. fistulosa*	χ^2= 3.192, p = 0.073

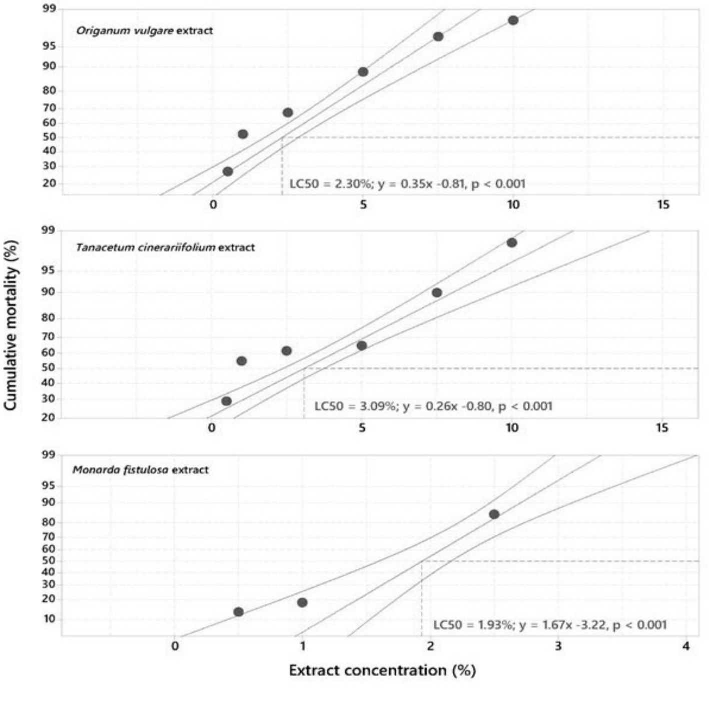

Fig. 2. Concentration–mortality response curves from the probit analyses testing insecticide effects of plant extracts against adults of *Ips typographus* in laboratory bioassays.

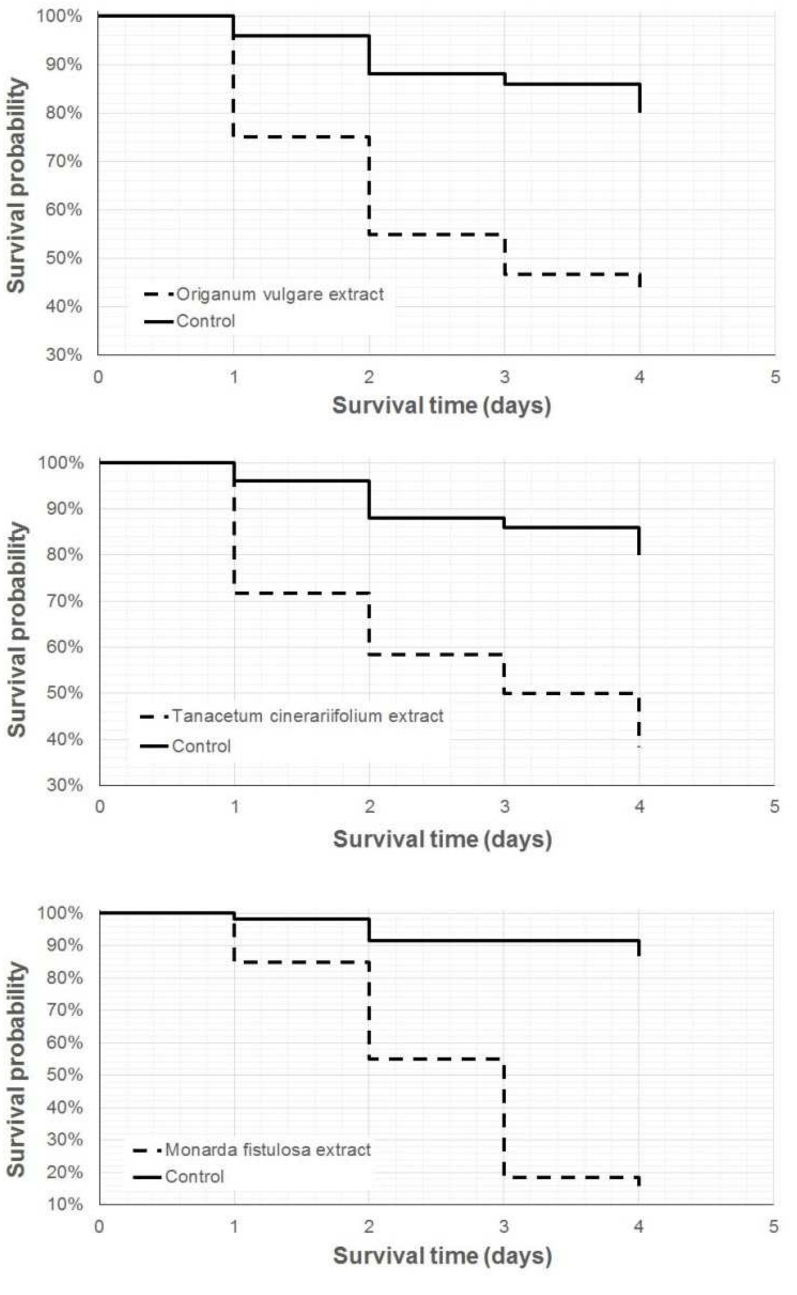

Fig. 3. Kaplan-Meier estimates of the survival curves for *Ips typographus* adults treated with the plant extracts (2.5%) in laboratory bioassays.

DISCUSSION

Many plants have evolved their own broad or insect-specific chemical defenses in an attempt to resist the destructive behavior of phytophagous insects. Humans have used these chemical compounds in crop protection with or without knowledge of the underlying chemicals and/or its mode of action on pests (Pavela 2016). Plants often have specifically evolved tissues, where the specialized metabolites are produced, stored and excreted. Their production is regulated by enzyme kinetics, precursor flux, feedback inhibition and is under a complex (post)-transcriptional control (Kortbeek et al. 2019). Although the first use of plant preparations as insecticides dates back to 2500–1500 BC (Oerke 2006), nowadays the properties of plant-based biopesticides - less toxic, target-specific, highly effective in small quantities, and biodegradable, make them alternatives to synthetic compounds in organic farming or integrated pest management approaches.

In recent years, following the growing interest in sustainable insect pest control methods, EOs have received increasing attention as components of natural chemicals. This is because, in addition to their properties against target organisms, they show high variability, low persistence and generally low toxicity to non-target animals (Isman 2006). However, knowledge on this issue, particularly on the side effects of EOs on beneficial insects, is still relatively limited (Tillman 2008). Recently, EOs are increasingly being used more widely as an alternative to conventional chemical control. EOs and their special constituents, mainly monoterpenoids, are considered promising alternatives to insecticides for controlling insect pests (Lopez et al. 2008; Tripathi et al. 2009; Abdelgaleil et al. 2016; Araújo et al. 2017). Mudronečková et al. (2019) evaluated the chemical composition of volatile extracts from six aromatic plants from the Lamiaceae and Apiaceae families. The authors concluded that while oxygenated monoterpenes predominated in *Mentha piperita* Linnaeus, 1753, *Pimpinella anisum*, Linnaeus, 1753, *Foeniculum vulgare* Mill, 1768 and *Hyssopus officinalis* Linnaeus, 1753 EOs, phenolic compounds dominated in *O. vulgare* and *T. vulgaris* EOs. Several authors determined the presence of differences in the composition of essential oils from several factors - environment, nutrition, plant growth conditions, genetic factors and the plant part from which they were extracted (Perry et al. 1999; Isman & Machial 2006; Nenaah 2014).

Origanum vulgare EO is known to produce an inhibitory effect on acetylcholinesterase and ATPase activities (Abdelgaleil et al. 2016) and its main constituent carvacrol (78.2%) exhibits mild inhibition of the acetylcholinesterase enzyme (Anderson & Coats 2012). Thymol (*Thymus vulgaris* Linnaeus 1753 EO; 50.4%) and carvacrol affect insect cells and cause disturbance in the cell membrane, causing it to lose its semi-permeable properties (Medeiros et al. 2011). Toshova et al. 2022 applied pure undissolved Greek oregano EO against *D. virgifera* against at a volume of 3, 5 and 10 µl caused rapid lethal effect (97–100% mean mortality). In the results the authors also demonstated that the treatment of WCR with four concentrations of EO (0.01, 0.1, 1 and 10 µl/ml) showed a strong concentration–time effect and mortality rate increased with increasing concentration and exposure time. The isomers - carvacrol and thymol - have been identified as the main components of the essential oils of *Satureja pilosa* Velen, 1811, *M. fistulosa* and *O. vulgare hirtum*. Carvacrol is a compound with previously demonstrated strong AChE inhibitory activity (Jukic et al. 2007) and probably determines the activity of the essential oils. The established profile of *O. vulgare hirtum* is consistent with literature data on the natural populations of the species in Bulgaria from the Eastern Rhodope Mountains, Struma Valley and cultivated areas (Konakchiev et al. 2004; Alekseeva et al. 2021; Baycheva & Dobreva 2021). The composition of *S. pilosa* and *M. fistulosa* corresponds to the report of Semerdjieva et al. (2020) and Ghosh et al. (2020), respectively. Maximum essential oil content can be found in inflorescences and leaves, and least in stems, and the main components of *M. fistulosa* essential oil are thymol and carvacrol (60–61%) (Casian et al. 2017). Essential oils from *M. fistulosa* and *Monarda bradburiana* Beck, 1826 have been previously studied for their repellent and larvicidal activity against *Aedes aegypti* (Linnaeus, 1762) thymol was detected as the most toxic compound followed by carvacrol, eugenol, and carvacrol methyl ether (Tabanca et al. 2013). Georgiev et al. (2022) reported for the first time the AChE activity of essential oils from *O. vulgare* ssp. *hirtum*, *T. longedentatus, S. pilosa* and *M. fistulosa*. They established a strong inhibitory activity of the tested essential oils as a prerequisite for the presence of insecticidal activity.

The toxicity of oregano EOs has also been documented against other coleopteran species in multiple studies (e.g. García et al. 2005; Kim et al. 2010; Saroukolai et al. 2010; Nenaah & Ibrahim 2011; Regnault-Roger et al. 2012; Yeom et al. 2012; Gonzalez-Coloma et al. 2013; Abdelgaleil et al. 2016; Mudrončeková et al. 2019) that show the effects of EO on *I. typographus*. Plant EOs and their individual metabolites have also demonstrated the potential for repellent activity against various insects (Kim et al. 2010; Nenaah 2014; Papachristos & Stamopoulos 2002). Our results confirm the presented earlier results by Mudrončeková et al. (2019), namely the fact that EOs show high contact toxicity and efficacy against *I. typographus* adults. Our results show a strong insecticidal effect of *M. fustulosa* EO, which is manifested by symptoms of slower movement and partial or complete paralysis of beetles, and subsequent death.

In their work Haider et al. (2015) presented the variations in composition and effect of EO of *Tanacetum nubigenum* Wallich *ex* DC, 1824 collected from several locations, at different altitudes. On the basis of their findings, it can be concluded that the potential application of EOs is directly related to their composition, which may differ depending on several factors, among which are: the qualities of the variety, the time of harvest, and the effects, caused by the environment.

Fierescu et al. (2020) claim that the insecticidal potential of EOs exceeds the potential of commercial synthetic insecticides at very low concentrations (typically <1% EO concentration). Noting that, based on the fact that most monoterpenes are toxic to animal tissues, a number of authors attribute the primary role in insecticidal action of the EO to these compounds. The mechanism by which ECs act is largely related to the method of administration: in direct contact, the most likely mechanism is through a neuronal toxicity (Mohanty & Jena, 2019); for fumigant application, the most likely mechanism is through the action of terpenoids on the respiratory system (Mossa 2016). The same author makes a connection between the mechanisms of repellent activity and variations in the olfactory receptors of insects.

The current authors anticipate that thymoquinone, p-cymene, thymol and carvacrol are some of the key components that (as demonstrated) show abundance and probably have an essential role intheinsecticidal activity of the tested *O. Vulgare* and *M. Fistulosa* essential oils. We hypothesize that the insecticide properties of *T. cinerariifolium* extract against the adults of the European spruce bark beetle can be associated with high contents of pyrethrins (33%) in the chemical profile. The observed strong contact toxicity in this study, as well as the typical symptoms of tremors, convulsions, paralysis, and the presence of subsequently high mortality of the treated insects, confirm the high sensitivity of *I. typographus* to the *T. cinerariifolium* extract.

CONCLUSION

The use of plant-based insecticides is not associated with risks such as negative environmental and health effects, pest resistance development, and negative effects on natural enemies. Based on our study, we can conclude that all three plant extracts have insecticidal activity against *Ips typographus*. Nevertheless, it is a subject of further research how such plant-based insecticides can be applied in nature.

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