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ORIGINAL ARTICLE

Exploring the bioactivity of a novel pine wood distillate (PWD) for plant growth and protection

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Abstract

Exploiting plants by-product properties for combined crop growth regulation and pest management could represent a strategy for a more sustainable agriculture. The present study investigated the potential activity (at different product concentrations) of a novel pine wood distillate (PWD) obtained as an industrial by-product, on multiple targets in the agroecosystem. In the weed species, *Sylibum marianum*, PWD stimulated a more than twofold increase of seed germination and seedling development, while it was able to inhibit by up to 70% the growth of the soil-borne plant pathogen *Fusarium culmorum* on durum wheat seedlings. PWD was also able to induce behavioural changes in mature females of the fruit fly *Ceratitis capitata*, with a significant reduction in the visit and oviposition rate on treated orange fruits (53% and 62% less, respectively). Analysis of PWD chemical composition suggested a role of phenolic compounds in the observed species-specific effects. Taken together, these results support a multivalent exploitation of wood distillates in the management of important crops of the Mediterranean area, aligning with both circular economy and environmental protection principles.

Keywords Biostimulants, weeds · Pest management · Wood extract · Plant pathogens · Biological control

Introduction

Reconsidering our agricultural production systems is a global priority to meet the food demand of a growing population and to safeguard the environment in both short and long perspective (Hartmann et al. 2015). Alongside a growing public awareness of environmental and food safety concerns, the legislative framework in major regions of the world is also evolving in this direction. Accordingly, European Union, with the European Green Deal, has targeted to increase organic farming to 25%, while reducing up to 50% the use of chemical pesticides by 2030 (European Commission 2019).

Similarly, removing or reducing the use of hazardous products remains a primary objective of the United Nations 2030 agenda for sustainable development, namely the Third goal: "Ensuring health and security for all and for all ages" (United Nations 2015). Though these goals are widely shared, there remains the need to ensure that effective, sustainable, and safe products could be available to the farmers to promote plant growth and to protect crops from biotic adversities such as weeds, plants pathogens and pests, which can severely compromise agricultural production, in a global context where invasive species often establish into new geographic areas (Deguine et al. 2023). This translates into the need to ensure satisfactory yields while ensuring the economic sustainability of the farm (Seufert et al. 2012).

Research towards the discovery and evaluation of novel bio-products is therefore the key to expand the availability of active substances that can be used within the agroecosystems with different purposes: management of pests and weeds or plant fertilization and stimulation (Barros

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et al. 2020). Moreover, the exploitation of those substances obtained from plant biomass by-products make possible the application of the circular bioeconomy approach, maximizing the reuse of materials within the same system while reducing inputs from non-renewable sources (Chen et al. 2020).

Indeed, biostimulants and biopesticides in the broadest sense (including bioherbicides), involving active substances of microbial and biochemical origin, represent the most concrete perspective, even if they are still available only against a limited range of targets (Glare et al. 2012). Microbials include plant growth promoters (Backer et al. 2018), entomopathogens (Ruiu 2018), and several plant pathogen antagonists (Heydari and Pessarakli 2010), while the biochemicals refers to bio-based substances that interact with the plant enhancing its growth capacity and health, or act against pests through non-toxic mechanisms (according to the definition of the United States Environmental Protection Agency) (EPA 2022). Among these last, there are essential oils, mainly consisting of terpene hydrocarbons or oxygenated compounds with both herbicidal and pesticidal activities (Giunti et al. 2021; Giannini et al. 2022); secondary metabolites such as glucosinolates which have suppressive effects against weeds (Matteo et al. 2018) and pathogens (Poveda et al. 2020). On the other hands, it is important to note that having a natural origin does not directly imply a safety profile for humans and non-target organisms (Fačkovcová et al. 2020).

Wood distillate, also known as pyroligneous acid (a by-product of plant biomass pyrolis), has been reported to have pesticidal and antimicrobial potential against lichens (Bianchi et al. 2022), insects (Urrutia et al. 2022), and pathogenic microorganisms (Riekkinen et al. 2022), as well as some biostimulatory effects on plant growth (Vannini et al. 2021). Despite considerable interest in the prospect of their large-scale use, the composition of these plant-derived products is complex, and their bioactivity potential and target range in the agroecosystem are still poorly understood (Grewal et al. 2018).

This study aimed to characterize a novel pine wood distillate obtained from industrial processing as a by-product, determining its composition, and assaying its biological activity on selected targets among crop, weed, pest and phytopathogen species occurring in the Mediterranean area.

Materials and methods

Pine wood distillate origin and composition

The pine wood distillate (PWD) used in this study was provided by Biologica Srl (Porto Torres, SS, Italy) as a by-product of industrial processing of *Pinus nigra* J.F. Arnold, obtained by gasification of pine wood. Briefly, plants were preliminarily chopped, pressed under anaerobic conditions, and then subjected to the core industrial process (700–800 °C without combustion). The main product obtained, called syngas, was washed with water to obtain the clean syngas (used as fuel) and the PWD as a by-product.

Physico-chemical analyses of PWD were conducted by Laboratorio Leonardi SAS (Porto Torres, SS, Italy), in compliance with ISO 9001:2015 recommendations, so as to determine pH, conductivity, colour, density, and the content in metals, organic compounds, and solvents. The content in potentially harmful pesticides and in polycyclic aromatic hydrocarbons (PAHs) was also determined. Standard protocols used for each analysis are reported in Table 1. According to Decision 2014/955/EU, Regulation 1357/2014/EU, and EU Regulation 1342/2014 with reference to the specific hazard codes, PWD had no hazardous characteristics since among the substances analysed and researched, classifiable as hazardous under the above-mentioned regulations, none exceeded the limit concentrations.

As a reference product in experiments with plant pathogens, a commercially available chestnut wood distillate (CWD) (BioDea, Arezzo, Italy) was used.

Seed germination and seedlings' growth experiment

Materials

Experiments with plants had the purpose to evaluate the biostimulant properties of PWD on a selection of cultivated species (*Eruca sativa* Mill. cv. NEMAT and *Vicia villosa* Roth cv Haymaker), and to determine a possible inhibitory potential against representative weeds (*Malva sylvestris* L. and *Silybum marianum* L.).

E. sativa (E) seeds were derived from the CREA-CI Brassicales collection (Lazzeri et al. 2013), while *V. villosa* (V) seeds were purchased from Padana Sementi (IT). *S. marianum* (S) seeds were collected from Porto Torres experimental field (Sassari, Italy) in summer 2018, while *M. sylvestris* (M) seeds were collected from Ottava experimental field (Sassari, Italy) in summer 2019.

Five solutions at 5 different PWD concentrations (C) were tested (C0: 0% v/v; C1: 0.6% v/v; C2: 1.25% v/v; C3: 2.5% v/v; C4: 5% v/v). The seeds of E, V, M and S were left soaking in the five stock solutions for 2 h. Then, seeds were rinsed with deionized water and placed in the Petri dishes (10 seeds each) equipped with double-layered Whatman No. 1 filter paper moistened with 2 ml of distilled water. Germination tests were carried out in complete darkness at a constant temperature of 23 °C (±2 °C) for 7 days.

Table 1 Physicochemical analysis of the pine wood extract (PWD) with indication of relevant official methods

Analysis	Method	DL	UM	Value	SD
pH	CNR-IRSA Manual 29/2003 2060		pH unit	2.35	±0.05
Conducibility	Reports ISTISAN 07/31 ISS.BDA.022.rev00	5	µS/cm	2119	±318
Color	–			Orange	
Density	–	0.1	kg/L	1.019	
Sedimentable solids	CNR-IRSA Manual 29/2003 2090	1	mg/L	<1.0	
Total suspended solids	CNR-IRSA Manual 29/2003 2090	5	mg/L	<5.0	
Metals					
Aluminum	EPA 6020	0.1	mg/L	1.97	±0.39
Arsenic	EPA 6020	0.0	mg/L	0.0220	±0.004
Barium	EPA 6020	0.1	mg/L	<0.10	
Boron	EPA 6020	0.1	mg/L	<0.10	
Cadmium	EPA 6020	0.005	mg/L	0.007	±0.001
Chromium	EPA 6020	0.1	mg/L	0.10	±0.02
Chromium VI	EPA 6020	0.1	mg/L	<0.10	
Iron	EPA 6020	0.1	mg/L	32.80	±6.56
Manganese	EPA 6020	0.1	mg/L	0.60	±0.12
Mercury	EPA 6020	0.001	mg/L	<0.001	
Nickel	EPA 6020	0.1	mg/L	0.41	±0.08
Lead	EPA 6020	0.1	mg/L	0.14	±0.03
Copper	EPA 6020	0.1	mg/L	<0.10	
Selenium	EPA 6020	0.005	mg/L	<0.005	
Tin	EPA 6020	0.1	mg/L	<0.10	
Zinc	EPA 6020	0.1	mg/L	3.96	±0.8
Organic compounds					
Phenols	CNR-IRSA Manuale 29/2003 5070	0.1	mg/L	3780.0	±756.0
Animal and vegetable fats and oils	CNR-IRSA Manuale 29/2003 5160	0.1	mg/L	5.4	±1.1
Mineral oils	CNR-IRSA Manuale 29/2003 5160	0.1	mg/L	<0.1	
Organic compounds of tin					
Dibutyltin	UNI EN ISO 17353:2006	0.01	mg/L	<0.01	
Monobutyltin	UNI EN ISO 17353:2006	0.01	mg/L	<0.01	
Tetrabutyltin	UNI EN ISO 17353:2006	0.01	mg/L	<0.01	
Tributyltin	UNI EN ISO 17353:2006	0.01	mg/L	<0.01	
Butyltin	UNI EN ISO 17353:2006	0.01	mg/L	<0.01	
Aromatic organic solvents					
Benzene	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Ethylbenzene	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
m-xylene	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
o-xylene	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
p-xylene	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Styrene	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Toluene	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Organic nitrogen solvents					
Acrylonitrile	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Ethyl methacrylate	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Malononitrile	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Methacrylonitrile	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Methylmethacrylate	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	
Propionitrile	EPA 5021A + EPA 8260B	0.01	mg/L	<0.01	

Table 1 (continued)

Analysis	Method	DL	UM	Value	SD
Chlorinated organic solvents					
1,1,1-trichloroethane	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
1,1,2,2-tetrachloroethane	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
1,1,2-trichloroethane	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
1,1-dichloroethane	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
1,1-dichloroethylene	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
1,2,3-trichloropropane	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
1,2-dichloroethane	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
1,2-dichloroethylene	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
1,2-dichloropropane	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
Vinyl chloride	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
Tetrachloroethylene	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
trichloroethylene	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
Trichloromethane (chloroform)	EPA 5021A + EPA 8260B	0.01	mg/L	< 0.01	
Total pesticides (excluding phosphorates)					
Aldrin	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Dieldrin	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Endrin	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Isodrin	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
2,4'-DDD	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
2,4'-DDE	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
2,4'-DDT	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
4,4'-DDD	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
4,4'-DDE	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
4,4'-DDT	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Alachlor	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Alpha-hexachlorocyclohexane	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Alpha-endosulfan II	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Atrazine	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Beta-hexachlorocyclohexane	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Beta-endosulfan I	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Cis-chlordane	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Delta-hexachlorocyclohexane	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Endosulfan sulfate	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Heptachlor	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Heptachlor epoxide	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Hexachlorobenzene	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Gamma-hexachlorocyclohexane	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Metoxychloro	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Trans chlordane	CNR-IRSA Manual 29/2003 5090	0.01	mg/L	< 0.01	
Phosphorus pesticides					
Azinfos metile	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
Clorfenvinfos II	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
Etion	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
Fention	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
Fosalone	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
Fosfamidone	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
Fosmet	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
Malation	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
Paration metile	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	

Table 1 (continued)

Analysis	Method	DL	UM	Value	SD
Tetraclorvinfos	CNR-IRSA Manual 29/2003 5100	0.005	mg/L	< 0.01	
PAH					
Benzo(a)anthracene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Benzo(a)pyrene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Benzo(b)fluoranthene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Benzo(k)fluoranthene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Benzo(g,h,i)perylene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Chrysene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Dibenzo(a,e)pyrene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Dibenzo(a,l)pyrene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Dibenzo(a,i)pyrene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Dibenzo(a,h)pyrene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Dibenzo(a,h)anthracene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Indenopyrene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	
Pyrene	EPA 3550C + EPA 8270D	0.10	mg/L	< 0.10	

DL detection limit, *UM* unit of measurement, *SD* standard deviation

Germination parameters and biometric traits

Germinated seeds were recorded daily and were counted when radicles were ≥ 2 mm in length. The following parameters were derived from the daily records: germination percentage (G), mean germination time (MGT), synchrony of the germination process (Z).

At the end of the experiment, additional parameters were recorded: the plant development percentage (PD), i.e. the number of developed seedlings over the total number of germinated seeds per Petri dish multiplied by 100; hypocotyl length (HL), main root length (RL), and the seedlings' fresh weight (SFW), all averaged to the number of seedlings per Petri dish.

These experiments were carried out according to a completely randomized design with 4 replicates per treatment. Each experiment was performed twice.

Antifungal activity bioassays

The activity of PWD on plant pathogens of the genus *Fusarium*, was evaluated on melon (*Cucumis melo* L.) and durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.).

Seeds of *C. melo* were preliminarily treated with 3% NaClO for 2 min, washed 5 times in sterile water, placed on 1.5% agar plates and incubated at 28 °C for 3 days. Seedlings were then transferred into 50 ml glass tubes containing 15 ml of growing medium (2.2 g/l Murashige and Skoog Basal Salt Mixture (MS), 1.5% agar, pH 5.8) and grown at 25 ± 2 °C for 48 h. Then, a conidia suspension of *Fusarium oxysporum* f. sp. *niveum* (isolate BET09, collection Bioecopest) Smith (FON) (10^4 conidia/ml) was inoculated (40 µl) into the medium, while PWD (0.2% v/v) was applied by root drenching just before transferring seedlings into the tubes.

Seeds of *T. turgidum* were treated with 3% NaClO for 2 min, followed by washing (5 times) in sterile water, before being placed on plates containing sterile water (15 ml) for 3 days. After germination, seedlings were transferred into 50 ml glass tubes containing 10 ml of the above described growing medium and incubated at 25 ± 2 °C. A conidia suspension (10^4 conidia/ml) of *Fusarium culmorum* (Wm.G.Sm.) Sacc. was directly inoculated (10 µl/tube) onto the medium and the PWD was applied (0.2%) according to the following two methods: (1) fungal inoculation 24 h after transplanting and direct inclusion of PWD into the growing medium; (2) fungal inoculation immediately after transplanting and PWD application by root drenching just before seedling transplanting and thus before the pathogen inoculation. Disease course was assessed daily for 21 days for melon and 13 days for durum wheat, reporting the degree of symptomatology severity in the scale of 0–3 (0 = no symptoms, 3 = plant death). These experiments were carried out with a randomized block design with three replications per treatment.

Interaction with insect pests

Insect bioassays were conducted on different stages of the Mediterranean fruit fly (Medfly) *Ceratitis capitata* Wied. (Diptera: Tephritidae) to detect possible toxicity and behavioural effects. This pest was selected as a study model, being a polyphagous species of relatively high and worldwide spread economic importance (White and Elson-Harris 1992).

Toxicity bioassays

Insect specimens used in this study were provided by the insect rearing facility of the Department of Agricultural Sciences of the University of Sassari (Italy) (Ruiu et al. 2015).

A first experiment was conducted on mature third instar larvae in the phase of searching for a suitable environment for pupation, which in nature can be represented by leaving the breeding fruit for pupation in the soil. For this purpose, larvae were immersed in the PWD solution (C1 = 1% v/v and C2 = 10% v/v) or sterile distilled water (C0 = control) for 30 s before being maintained in groups of ten individuals inside Petri dishes (3.5 cm diameter) in an incubator at 25 ± 1 °C to allow pupal development till adult emergence. The rates of pupated insects (P) and of emerged adults (AE) calculated on the number of treated larvae, were recorded. This experiment had a completely randomized design with five replicates and was repeated three times with different cohorts of larvae.

Behavior bioassays

A second experiment, conducted on adults at 25 ± 1 °C at a photoperiod of L14:D10, had the purpose to study the possible effects on the oviposition behaviour, evaluating the occurrence of female visits and their oviposition rate on PWD-treated compared to water-treated fruits. Accordingly, navel orange fruits (*Citrus sinensis* (L.) Osbeck cultivar Washington Navel) with a diameter of 10 cm were offered to newly emerged and just mated fruit fly females obtained from a laboratory mating cage in which they were in contact with males for 5 days after emergence (Falchi et al. 2015) The experimental design involved groups of 5 mated females in a Plexiglas cage (30 by 30 by 30 cm) with two windows covered with gauze, to which water and saccharose were provided ad libitum. A no-choice experimental design was followed, involving a single fruit treated with PWD or water (control) maintained in each cage with 5 females for 48 h. During this period the following parameters were monitored: (1) number of female visits/fruit (NV) (1 min observation every hour for 8 h during the day recording the number of females landing on fruits); (2) number of oviposition punctures/fruit (OP) (counted under a stereomicroscope after 48 h exposure, verifying the presence of fly eggs). PWD was applied using a hand sprayer (10 ml per fruit) at concentrations of 0.0% v/v (C0), 0.5% v/v (C1), and 5% v/v (C2). The experiment had a completely randomized design involving five replications, and was repeated twice with different cohorts of females.

Statistical analysis

All statistical analyses were performed in the RStudio application of R software (R Core Team, 2014) environment.

Packages lme4, emmeans, multicomp were used for analyses of seedling growth parameters. Given the heteroscedasticity of the parameters after Bartlett's test, G, PD and Z were processed using a generalized linear model with a quasi-binomial distribution using a logit link function, while all the other parameters were processed using a generalized linear model with a quasi-Poisson distribution using a logit link function. The two experiments were analyzed separately, and each was analyzed by one-way ANOVA. The significance of the differences between the mean values of the treatments was evaluated using Tukey's test at $P < 0.05$.

Data on insect pupation, adult emergence, number of visits and oviposition punctures per fruit were analysed by 1-way ANOVA followed by Tukey HSD test for post-hoc comparison of means.

Data on plant pathogen symptomatology severity were analysed by Pairwise Wilcoxon Rank Sum Test (R-package stats v3.6.2).

Results

l Effects on seeds and seedlings of cultivated plants and weeds

The selected cultivated and weed species were differently affected by the treatments with PWD (Table 2).

In *Sylibum marianum* (S), PWD treatments affected both germination and seed development. Indeed, both G and PD were significantly higher in all the treatments with PWD than under C0. Even the synchronization of seed germination (Z) changed according to treatment with the highest value under C3. About the biometry of seedlings, only NLR and HL showed some statistically significant differences. The highest HL was detected under C3, while the lowest under C4. About NLR, the highest value was recorded under C1 and it was three times greater than under control (C0).

In *Vicia villosa* (V), only two germination parameters were influenced by treatments: PD and Z. The highest PD was detected under C0, strictly followed by C3 and then C1 and C4, with the lowest value under C2. Differently, the highest Z was found under C1 followed by C0 and then all the other treatments for which the value was almost similar.

On the other hands, germination process and seedlings growth in the other two target seeds, *Eruca sativa* and *Malva sylvestris,* were not significantly influenced by the treatments. In *Eruca*, the only difference was observed in NLR which was significantly reduced from C0 to the other treatments in which was equal to 0 (Table 2).

Table 2 Mean separation among the treatments for each plant species

Treatments	G (%)	PD (%)	Z	MGT (day)	RL (cm)	HL (cm)	SFW (g)	NLR
Silybum marianum (S)								
C0	44a	41a	0.65a	2.20a	2.84a	2.20ab	0.14a	0.81a
C1	89b	86b	0.82ab	2.19a	3.85a	2.26ab	0.12a	2.24b
C2	90b	82b	0.81ab	2.14a	3.35a	2.17ab	0.13a	1.97ab
C3	97b	82b	0.95b	2.02a	3.05a	2.39b	0.12a	1.75ab
C4	91b	89b	0.76ab	2.17a	2.55a	1.61a	0.10a	1.11ab
Eruca sativa (E)								
C0	100b	80a	0.88a	1.07a	2.42b	1.78b	0.018a	0.22b
C1	100b	77a	0.9a	1.05a	1.36a	0.56a	0.015a	0a
C2	85a	65a	0.78a	1.11a	1.37a	0.56a	0.017a	0a
C3	87a	67a	0.72a	1.20a	1.31a	0.53a	0.024a	0a
C4	85a	70a	0.72a	1.15a	2.01ab	0.69a	0.018a	0a
Vicia villosa (V)								
C0	73a	73b	0.54ab	2.55a	3.95a	6.10a	0.18 a	2.24a
C1	57a	57ab	1b	2.67a	3.28a	6.96a	0.17a	2.63a
C2	42a	40a	0.30a	2.80a	3.57a	5.59a	0.16a	1.35a
C3	75a	72ab	0.35a	2.71a	3.41a	6.04a	0.18a	2.41a
C4	57a	55ab	0.30a	2.92a	2.86a	5.23a	0.16a	1.81a
Malva sylvestris (M)								
C0	95a	87a	0.67a	1.37a	2.99a	4.91a	0.06a	1.63a
C1	97a	88a	0.52a	1.44a	3.21a	5.14a	0.06a	1.57a
C2	90a	80a	0.72 a	1.21a	2.73a	4.89a	0.06a	1.54a
C3	92a	81a	0.59a	1.36a	2.74a	4.87a	0.06a	1.17a
C4	92a	82a	0.54a	1.42a	2.85a	4.64a	0.06a	1.04a

Different combinations of lowercase letters indicate significantly differing means ($P < 0.05$, Tukey's test)

The treatments were (C0: 0% v/v; C1: 0.6% v/v; C2: 1.25% v/v; C3: 2.5% v/v; C4: 5% v/v)

G germination percentage, *PD* plant development percentage, *Z* synchrony of the germination process, *MGT* mean germination time, *RL* main root length, *HL* hypocotyl length, *SFW* seedlings' fresh weight, *NLR* number of lateral roots

Effects of PWD on soilborne plant pathogens

Application of PWD on durum wheat seedlings was able to supress fungal activity of *F. culmorum,* artificially inoculated after the product application (Fig. 1a). Indeed, the disease severity score (scale 0–3) at 13 days post inoculation (dpi) was significantly lower for PWD treated plants ($P < 0.05$, Wilcoxon rank sum test), compared to control

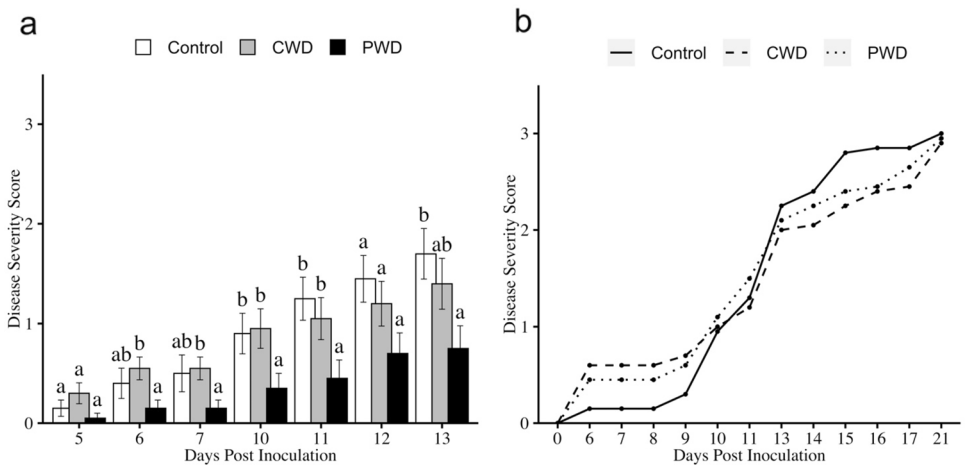

Fig. 1 Effects of wood distillates on *Fusarium*-induced diseases. Disease severity score, reported on a scale of 0 to 3 (0 = no symptoms, 3 = plant dead), assessed on **a** *F. culmorum*-infected durum wheat plants, with different combinations of lowercase letters indicating significantly differing means ($P < 0.05$, Wilcoxon rank sum test) and **b** FON-infected melon plants, with no significant difference reported. (*CWD* chestnut wood distillate, 0.2% v/v; *PWD* pine wood distillate, 0.2% v/v)

Table 3 Mean (±s.d.) separation among the treatments for *Ceratitis capitata* pupation and adult emergence rates

Treatments	*P*	AE%
C0	97.3 ± 4.6a	88.7 ± 7.4a
C1	92.7 ± 8.0a	82.0 ± 6.8a
C2	94.7 ± 7.4a	71.3 ± 11.3b

Different combinations of lowercase letters indicate significantly differing means ($P < 0.05$, Tukey's HSD test). (C0: 0.0%; C1: 1%; C2:10%; *P* pupation rate, *AE* adult emergence rate)

Table 4 Mean (±s.d.) separation among the treatments for *Ceratitis capitata* female visits and oviposition punctures rates on fruits

Treatments	NV	OP
C0	17.2 ± 1.8a	9.4 ± 1.3a
C1	14.6 ± 2.9b	9.2 ± 1.4a
C2	9.1 ± 1.4c	5.8 ± 1.2b

Different combinations of lowercase letters indicate significantly differing means ($P < 0.05$, Tukey's HSD test). (C0: 0.0%; C1: 0.5%; C2: 5%; *NV* number of visits/fruits, *OP* oviposition punctures/fruit)

and CWD treated plants, reporting values of 0.75, 1.7 and 1.4 respectively. The disease suppression reported in PWD treated plants started at 6 dpi and the difference with the control ranged from 51% (12 dpi) to 70% (7 dpi), suggesting both a fast and persistent effect of PWD in reducing the disease severity.

In contrast, PWD did not affect *F. oxysporum* f. sp. *niveum* pathogenic activity (Fig. 1b). The product was applied to 5-days-old melon seedlings and early symptoms were detected at 6 dpi. No significant difference was reported at any timepoint in this case and all the plants were dead at 21 dpi.

Effects on survival and behaviour of Medfly

The results of PWD treatments to the third-instar larvae of *C. capitata* are shown in Table 3. No significant mortality was observed in the treated larvae that successfully pupated, as was the case with the control ($F_{2,42} = 1.76$; $p = 0.1842$). The resulting adult emergence rates were slightly, though significantly, reduced in pupae from larvae treated with the highest concentration (C2), compared with control (C0) ($F_{2,42} = 15.11$; $P < 0.001$).

The mean number of visits ($F_{2,27} = 36.97$; $P < 0.001$) and oviposition punctures ($F_{2,27} = 24.24$; $P < 0.001$) per fruit were significantly affected by treatments with PWD. As shown in Table 4, the number of visits in the fruits treated with the highest concentration was about half that of the control treated with water. Similarly, an almost 40% reduction in oviposition punctures was observed in fruits treated with PWD at a concentration of 5% v/v (C2).

Discussion

The experimental results revealed that the application of PWD, at different concentrations on diverse targets, can generate both biostimulant and biocidal activity.

The plant seeds used in this study were not significantly affected by treatments with PWD, with the sole exception of *Silybum marianum* that resulted to have greater germination and plant development percentages as well as longer hypocotyle length. This finding arising from our test on a selected number of species, seemed to be in contrast with large literature reporting inhibitory effects of phenolic compounds on seed germination and growth (Williams and Hoagland 1982; Macías et al. 2019). Nevertheless, the response to specific compound is absolutely species-specific (Giannini et al. 2021). Indeed, Reigosa and Souto (1999) reported that the application of different phenolic compounds on six weed seeds inhibited their germination and development with the exception of *Cirsium* sp. that was stimulated by gallic acid application. The extract used in the present study, namely PWD, contains 3780 mg/l of phenols as reported in Table 1, and the only species that was promoted by its application was *Silybum marianum*, which is phylogenetically closed to *Cirsium* (Barres et al. 2013).

Experiments on selected plant pathogen and insect species indicate a potential of the pine wood distillate as plant protection product against pests affecting relevant crops in the Mediterranean area. Indeed, PWD was able to significantly contrast *F. culmorum* in durum wheat seedlings and to interfere with the behaviour of *C. capitata* ovipositing females, which tended to visit less and lay fewer eggs in orange fruits.

F. culmorum and *C. capitata* are two major challenging pests in agriculture and this result may pave the way toward new approaches for soil borne plant pathogens and insects management, indicating PWD as potential natural product against some of the most serious problems in cereals and citrus (Wagacha and Muthomi 2007; Ganie et al. 2022).

In contrast, PWD was totally ineffective in reducing the disease severity in FON-inoculated melon seedlings, as well as no significant direct toxic effects of PWD (at standard concentration of 0.2%) were observed on *C. capitata* larvae, on which only a slight decrease in adult emergence was recorded following treatment at the highest concentrations assayed (10%). This may depend on the strict specificity of PWD, which could be due both to a different direct toxicity against different pest species and to a variable interaction with the plant toward which, in addition to the aforementioned growth promotion effects, a stimulation of its defence response may be determined

(Daayf et al. 2012). The absence of specific toxins in the chemical composition of PWD, including possible pesticide residues, suggests a potential role of phenolic components, known to have antimicrobial and antioxidant properties, and to interact with the plant-defensive mechanisms (Kumar et al. 2020).

Finally, experiments on plant pathogens provide evidence of different antimicrobial activity between CWD and PWD. Although both products were obtained through the same industrial process, only the latter showed significant effects against *Fusarium*. Given that such distillates are derived from wood of different plant species (pine for PWD and chestnut for CWD), we can assume that the differences in their biological properties are attributable to a diverse chemical composition, which is known to be closely related to the wood type, in addition to the distillation system (Rodríguez Madrera et al. 2003).

Conclusions

The present study evaluated from a multiple perspective the effects of a new pine wood distillate (PWD) obtained as an industrial by-product on different agroecosystem actors, namely crop plants, weeds, phytopathogens and insects. The results showed potentialities on several fronts albeit with effects that appear species-specific. Everything considered, a promising outlook is thus emerging for the employment of wood distillates in agriculture (normally used as corroborants), which opens new avenues for research in this field and future practical applications, aligning with both circular economy and environmental protection principles.

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Declaration

Competing interest The authors report no declarations of interest.

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