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A RESEARCH ON THE USAGE OF EXTRACTS FROM TWO POISONOUS PLANTS (*MUSCARI NEGLECTUM* GUSS. AND *GYNANDRIRIS SISYRINCHIUM* (L.) PARL.) AS A WOOD PRESERVATIVE

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ABSTRACT

In this study, the antifungal property of poisonous plant extracts from *Muscari neglectum* Guss. and *Gynandriris sisyrinchium* (L.) Parl. were determined when being evaluated as wood preservatives. Mixtures of bulb and leaves of the plants mentioned above were individually extracted using ethyl alcohol. Wood blocks of Turkish oriental beech (*Fagus orientalis* L.*)* and Scots pine (*Pinus sylvestris* L.) were impregnated with the plant extracts. The abilities of the extracts to suppress attack by *Postia placenta* (Fr.) (a brown-rot) and *Trametes versicolor* (L: Fr.) Quel. (a white-rot) were investigated. Treated blocks were exposed to *P. placenta* and *T. versicolor* attacks for 12 weeks by following the soil block method. While untreated wood specimens have weight loss ranging between 35.06 and 41.71 % for *P. placenta,* and 12.00 and 30.29 % for *T.versicolor.* The wood treated with the plant extracts is of weight loss between 5.38 and 18.30 % for *P. placenta*, and between 7.34 and 42.76 % for *T. versicolor.* The lowest weight loss (5.38 %) was found to be for beech wood impregnated with the extract of *Gynandriris sisyrinchium* at a concentration level of 0.25 % against *P. placenta.* Meanwhile, the highest weight loss was also on the beech wood (42.76 %) treated with the same plant extract at the level of 3.00 % concentration against *T. versicolor.*

KEY WORDS: Poisonous plant extracts, *Gynandriris sisyrinchium* (L.) Parl., *Muscari neglectum* Guss., *Postia placenta*, *Trametes versicolor*

INTRODUCTION

Owing to the special properties of wood, it has remained one of the most important renewable natural resources available to humankind throughout the course of history (Hingston et al. 2001, Wang et al. 2005). However, timbers that are not naturally durable are treated with preservatives to prevent decay by wood-boring crustaceans and molluscs, and fungi and insect attack (Yalinkilic, 2000, Craig et al. 2001). When timber is used as a construction material, it is generally treated with a chemical preservative to prevent damage by these aggressive biodeteriogens (Craig et al. 2001). Therefore developing methods to prolong the service life of wood has always been the interest of wood industry researchers (Wang et al. 2005).

A large number of preservative compounds have been introduced on to the market; however many of them not gained acceptance either because of chemical toxicity, low efficacy, high cost, or corrosiveness (Murphy 1990). Some contaminants are potentially included in wood preservatives, such as chromated copper arsenate (CCA), arsenic, creosote consisting of various polycyclic aromatic hydrocarbons (PAHs), chlorophenols (CPs) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs) as impurities in CPs, and organochlorine insecticides such as drin compounds and chlordane compounds (Sakai et al. 2001). Waste wood has the potential to include such chemicals which are considered to be persistent pollutants that have raised concerns about health effects on humans and wildlife (Asari et al. 2004). The persistence of these chemicals in the environment has resulted in their widespread existence throughout the food chain (Margaret et al. 1999, Wang et al. 2001).

From the environmental perspective, development of more environmentally acceptable preservatives, is a priority for the wood preservation industry today, and has led to a renewed interest in natural-based wood preservatives which have lower mammalian toxicity than many other wood preservatives (Ozaki 1999, Tagboto and Townson 2001). The ability of wood and natural plant extractives to protect wood against wood degrading fungi and insects have been one possible approach for developing new wood preservatives (Sen et al. 2002, Kartal et al. 2004, Schultz and Nicholas 2000). Therefore, in recent years, Wood Preservation Industry prefers non-chemical based and vegetable based chemicals for wood treatments. For example, Onuorah (2000) reported the effectiveness of the heartwood extracts of *Milicia exelsa* (Wlev.) and *Erythrophelum suaveolens* (Guil.& Perr.) to suppress attack by *Lenzites trabea* or by *Polyporous versicolor.* Chang et al. (1998) reported that α-cadinol has demonstrated to possess the highest antifungal effectiveness, which obtained from Taiwania heartwood. Konodo and Imamura (1986) had also investigated the antifungal compounds in heartwood extractives of *Chamaecyparis obtuse.* Digrak et al. (1999) studied the antimicrobial activities of valex (the extract of valonia), the extracts of mimosa bark, gallnut powders, *Salvia aucheri* Bentham var. *aucheri* and *Phlomis bourgei* Boiss. Goktas et al. (2006) studied the ability of *Sternbergia candida (SB Candidum* Mathew.*)* extract to suppress attack by *Postia placenta* and *Trametes versicolor.* Yang et al. (2007) reported that the ethanolic extracts from the bark of *Acacia confuse* exhibited strong antioxidant activity. Yang and Clausen (2007) investigated antifungal effect of seven essential oils derived-ajowan, dill weed, *Egyptian geranium*, lemongrass, rosemary, tea tree, and thyme. Goktas et al. (2006) studied antifungal activities of wood treated with aqueous solutions of *Juglans regia* extract. Schultz and Nicholas (2000) reported that heartwood extractives may be alternative wood preservatives as they have fungicidal and antioxidant properties. The results of these studies indicated that some natural extracts had the antibacterial and antifungal activity and can be used as wood preservatives.

The objective of this study was to determine the efficacy of natural poisonous plant extracts from *Muscari neglectum* Guss. and *Gynandriris sisyrinchium* (L.) Parl., in suppressing *Postia placenta* and *Trametes versicolor* attacks to treated sapwood of Turkish oriental beech (*Fagus orientalis* L.*)* and Scots pine (*Pinus sylvestris* L.).

MATERIAL AND METHODS

Material

Extracts used for study were obtained from *Muscari neglectum* Guss. and *Gynandriris sisyrinchium* (L.) Parl. bulbs and leaves. They were collected in Mugla-Yilanli Mountain with an altitude of 1275 m, and in Mugla-Yesilyurt-Dagdibi with an altitude of 500–550 m, respectively. The collected samples were air dried and kept in the Herbarium of Mugla University-Turkey.

For the impregnation process, wood specimens [19 (tangential) x 19 (radial) x 19 (longitudinal) mm] were prepared from the air-dried sapwood of Turkish oriental beech *(Fagus orientalis* L.) and Scots pine (*Pinus sylvestris* L.).

Postia placenta (Fr.), M. Larsen et Lombard (Mad 698), (a brown-rot) and *Trametes versicolor* (L: Fr.) Quel. (FFPRI 1030: Fungal accession number of Forestry and Forest Products Research Institute, Tsukuba, Japan) (a white-rot) were used as wood-decaying fungi.

Preparation of extract

A mixture of bulb and leaves of the plants was ground into particles with 1–2 mm, blended with 100 ml ethyl alcohol for each trial and placed into an alcohol bath at 50°C for 5 h. The resultant extract solution was filtered through a glass wool filter and then rinsed with a small quantity (about 30 ml) of 96 % ethyl alcohol. The extract solutions were evaporated to constant weight under reduced pressure at 50°C. Subsequently, the extracts were diluted with distilled water and frozen in a deep freezer at -4°C temperature. Then, the frozen extract was freeze dried to separate the more volatile extracts. The resulting extracts were kept in deep freezer at -20°C for later usage.

Impregnation of wood specimens with plant extracts

Air-dried wood specimens were impregnated with plant the extracts, under vacuum using a vacuum desiccator. Vacuum was applied for 30 min at 760 mm Hg^{-1} before introducing an extract into the vacuum chamber followed by another 30 min at 760 mm Hg^{-1} as a diffusion period under vacuum. The carrier solvent used was 100 ml distilled water for each extract concentration (0.25 %, 0.75 %, and 3.00 %). The total number of treated specimens was 160, providing 5 replicate by 4 different concentration levels, by 2 plant extracts, by 2 different sapwood specimens by 2 fungi. The impregnated wood specimens were removed from the chamber, wiped, weighed (T_2), air dried for one week at ambient temperatures, and then dried in an oven at 60° C for 3 days. For calculation of the amount of preservative absorbed by wood specimen, that is the retention, as kilograms per cubic meter ($kg.m^3$) were as follows:

$$\text{Retention} = (G \times C/V) \times 10\ kg.m^{-3} \quad (1)$$

Where;
$G = (T_2 - T_1)$ = amount grams of treating solution absorbed by the wood specimen (g)
T_1 = initial weight of the conditioned wood specimen before impregnation (g)
T_2 = weight of the wood specimen immediately after impregnation and wiping (g)
C = concentration of treatment solutions (%)
V = volume of wood specimens (cm^3)

Decay test

The treated and untreated wood specimens to be used for decay test were singly placed on trays and exposed to room conditions for 72 hours. Afterwards, all the wood blocks were placed in a conditioning chamber for 21 days to enable them to achieve equilibrium moisture content (EMC) of 12 %.

Wood samples were decayed in a soil-block procedure according to modified ASTM Standard D-1413 (1999). Fungal culture bottles were filled with 120 cm^3 of soil mixture and 62 g of distilled water were added to each bottle. The amounts of water to be added was calculated according to the "Water Hold Capacity" and "Water Required" equations from ASTM D-1413 (1999). Bottles were sterilized in an autoclave for 30 min at 121°C. After the sterilization soil culture bottles were thoroughly cooled. Fungal inoculum samples measuring 10mm in diameter were taken from a Petri dish culture. The inoculum was placed in contact with an edge of the feeder strip in the soil culture bottles. The soil cultures were incubated at 27 °C and 72.0 % relative humidity for 3 weeks. Then wood specimens were subjected to a modified decay resistance test. Five replicates of each treated wood specimen were dried to constant weight and steam-sterilized at 100 ± 2°C for 20 min. After cooling, the sterilized blocks were set in the bottles under air laminar flow conditions to avoid contamination. Bottle tops were screwed on and loosened one-quarter turn. Then, they were exposed to *Postia placenta* and *Trametes versicolor* fungi. The soil block cultures were incubated at 27°C with 75 % relative humidity for 12 weeks. At the end of the incubation period, wood specimens were removed from the test bottles and the mycelium was carefully brushed off the samples. Test specimens were then re-weighed after 4 weeks of conditioning in order to reach EMC , in the open laboratory (T_4). Weight losses were calculated from the conditioned weight of the wood specimen immediately before and after testing as follows**:**

$$\text{Weight loss (\%)} = (100\ (T_3 - T_4)\ /\ T_3) \quad (2)$$

where,
T_3 = weight of wood specimen plus remaining preservative after conditioning and before exposure to the test fungi (g),
T_4 = weight of the wood specimen after test and after final conditioning (g).

RESULTS AND DISCUSSION

The percent weight loss of treated wood specimens caused by *P. placenta* and *T. versicolor* after 12 weeks exposure and the results of statistical analyses are presented in Tab 1. Test results evaluated statistically by "Dunnett t" method that it tests treat one group as a control, and compare all other groups against it. The method reveals that beech and Scots pine wood impregnated by *Muscari neglectum* Guss. and *Gynandriris sisyrinchium* (L.)

extracts at all retention levels were effective in suppressing the attack of *Postia placenta*. Also, beech wood impregnated at retention levels of 2.61 kg.m^{-3}, and Scots pine wood impregnated at retention levels of 2.70 kg.m^{-3} and 6.37 kg.m^{-3} with *Muscari neglectum* Guss. were effective in preventing attack against *Trametes versicolor*. However, extracts of *Gynandriris sisyrinchium* (L.) were effective at a retention of 1.51 kg.m^{-3} on beech and at a retention of 1.11 kg.m^{-3} on Scots pine wood against *Trametes versicolor*.

Tab. 1: Weight loss of treated wood specimens after 12 weeks exposure to fungi

Wood species	Treatment	Concentration (%)	Retention level (kg .m^{-3})	Weight loss[a] (%)	
				Species of fungus	
				Postia placenta *Mean* ± SD	*Trametes versicolor* *Mean* ± SD
Turkish oriental beech (*Fagus orientalis* L)		**Control**	**0.00**	**35.06±3.64**	**30.29±3.20**
	Muscari neglectum Guss.	0.25	0.98	18.30±1.34*	41.85±0.64 NS
		0.75	2.61	6.18±0.19*	7.50±0.50*
		3.00	8.50	6.07±0.57*	40.02±3.26 NS
	Gynandriris sisyrinschium (L.) Parl.	0.25	0.50	5.38±0.09*	28.71±1.49 NS
		0.75	1.51	6.27±0.30*	26.52±0.52*
		3.00	7.20	6.38±0.65*	42.76±1.17 *b
Scots pine (*Pinus sylvestris* L)		**Control**	**0.00**	**41.71±3.88**	**12.00±0.95**
	Muscari neglectum Guss.	0.25	0.71	6.18±0.21*	12.44±0.77 NS
		0.75	2.70	6.03±0.33*	7.34±1.53*
		3.00	6.37	6.18±0.48*	10.57±0.29 NS
	Gynandriris sisyrinschium (L.) Parl.	0.25	1.11	6.45±0.55*	10.75±0.46*
		0.75	1.91	6.51±0.16*	16.41±0.67 *b
		3.00	9.63	6.52±0.11*	23.96±0.47 *b

* The mean difference is significant at the, 05 level.
[a] Dunnett t-tests treat one group as a control, and compare all other groups against it.
NS: Non significant
SD: Standard deviation
[b] There is a positive effect of the extract on the fungus that feeds it.

It is also clear from Tab. 1 that the lowest weight loss value (5.38 %) was obtained for the beech wood treated with *Gynandriris sisyrinchium* (L.) extract at retention of 0.50 kg.m^{-3} against *Postia placenta*. There are large amounts of phenol-glucosidic compounds in the bulbs of the *Gynandriris sisyrinchium* (L.) Parl. (Damirov et. al. 1996, Rahmana et al. 2003) which inhibited the growth of fungus *Postia placenta* on beech wood and Scots pine. But the highest weight loss value (42.76 %) was obtained for beech wood treated with *Gynandriris sisyrinchium* (L.) extract of 3.00 % concentration against *Trametes versicolor*. The extract of *Gynandriris sisyrinschium* in 3.00 % concentrations on beech and the same extract in 0.75 % and 3.00 % on pine may be exhibiting nutritive properties for *Trametes versicolor*. It can be seen as a contradiction which the efficacy of the extract is found to decrease with increasing concentration levels. Because the species of Muscari Mill. and Gynandriris (L.), contain pirrolizidin alcoholoids (1,2-dihydroxy-3-hydroxymethylpyrrolizidin) (Asano et al. 2002). These alcoholoids combine the tree fungus' enzymes that digest the cellulose and inhibit it. This happens in the low

concentrations of the plant extracts (*Muscari neglectum* Guss. and *Gynandriris sisyrinschium* (L.)). However, at high concentrations, the amount of nutritive material may increase. Because in the high concentrations, the monosaccharides covers the fungus and do not let the alcoholoid to inhibit the cellulose digesting enzyme. This cause the increase of the enzymes and funguses affects the plants. On the other hand, the monosaccharide provides sources of food for fungus. The pirolizidin alcoholoids and homo isoflavonoides are both effective in these process. The efficiency of *Muscari neglectum* Guss. against the fungi studied here can result from homo-isoflavonoides group in the extract, such as 3-benzylchroman–4–ones, flavons type compounds. This type of flavones has antiviral, antioxidant and antifungal effects (Barone et al. 1988, Cakir et al. 2003). There are large amounts of phenol-glucosidic compounds in the bulbs of the Gynandriris sisyrinchium (L.) Parl. (Damirov et. al. 1996, Rahmana et al. 2003) which inhibited the growth of fungus *Postia placenta* on beech wood and Scots pine.

CONCLUSION

Wood blocks of Turkish oriental beech (*Fagus orientalis* L.*)* and Scots pine (*Pinus sylvestris* L.) were impregnated with extracts from *Muscari neglectum* Guss. and *Gynandriris sisyrinchium* (L.) Parl. The effects of the extracts on the developments of *Postia placenta* (Fr.) (a brown-rot) and *Trametes versicolor* (L: Fr.) Quel. (a white-rot) were studied. The lowest weight loss was found to be for beech wood (5.38 %) which impregnated with the extract of *Gynandriris sisyrinchium* at a concentration level of 0.25 % against *P. placenta*. Meanwhile, the highest weight loss was also on the beech wood (42.76 %) treated with plant the same extract at the level of 3.00 % concentration against *T. versicolor*. The both extracts did a good job of inhibiting to *P. placenta* on beech and Scots pine wood, but no other parameters were evaluated (such as leaching) so, it is still a bit early to consider this extract as effective preservative.

Furthermore, the intention of the development of more environmentally friendly wood preservatives should encourage scientists to open the door to use plant extracts as wood preservatives because the plant extracts can offer substantial advantages for wood protection, providing sufficient decay resistance against fungi at low cost, low mammalian toxicity, and ease of handling and treatment.

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