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Effect of plant extracts on *Trametes versicolor* (White rot) fungal colonization and inhibition of treated wood samples of *Pinus roxburghii* Sargent

RK Meena, Bhupender Dutt, KR Sharma, JN Sharma and Rajneesh Kumar

Abstract
The present studies were conducted on effect of plant extracts on *Trametes versicolor* (White rot) fungal colonization and inhibition of treated and untreated wood of *Pinus roxburghii* Sargent. The data on colonization and fungal inhibition were noticed significant variation. Among the concentrations, maximum value for fungal colonization treated wood samples with extract was noticed in the (100%) for the controlled wood samples and minimum was found in 2.00% concentration (12.86%).The maximum colonization was observed in the *Acorus calamus* extract treated wood samples (59.04) and minimum in *Parthenium hysterophorus* treated extracts wood samples. In case of solvent used for the extraction, maximum fungal colonization was recorded in petroleum ether extract (58.69%) treated wood samples and minimum (53.38%) in methanol extract treated samples. In case of plant extract and chromium treated wood samples. The data for concentrations, maximum value (76.47%) was found in controlled wood samples and minimum in (10.31%) 2.00% concentration. The maximum value (49.31%) for the extract was noticed in *A. Calamus* treated wood samples and minimum value (46.84%) in *P. hysterophorus* treated wood samples. Among the solvent used for extraction maximum value (48.84%) showed in petroleum ether extract treated samples and minimum (47.31%) in methanol extract treated samples. Among the concentrations, maximum value (87.19%) for inhibition treated wood samples with extract was found in the 2.00% concentration treated wood samples and minimum 0.25% concentration. The maximum inhibition was noticed in the *P. hysterophorus* extract treated wood samples (56.37%) and minimum (49.27%) in the *A. calamus* extracts treated wood samples. In case of solvent used for the extraction, maximum inhibition was noticed in methanol extract (56.06%) treated wood samples and minimum (49.57%) in petroleum ether extract treated samples. In case of plant extract and chromium treated wood samples. The data for concentration, maximum value (90.41%) was observed in 2.00% concentration and minimum in (10.44%) 0.25% concentration. The maximum value (49.44%) for the extract was noticed in *P. hysterophorus* treated wood samples and minimum value (43.75%) in *A. Calamus* treated wood samples. Among the solvent used for extraction maximum value (49.81%) showed in petroleum ether extract treated samples and minimum (43.37) in methanol extract treated samples.

Keywords: Plant extracts, *Trametes versicolor*, wood, wood durability, Solvents

Introduction
Wood is one of the most vital natural resource on earth, and plays an important role in the world economy. Presently, the demand for quality timber and wood based products is very high and is increasing with time. To meet this increasing demand, efficient use of timber species is of key importance, there is heavy pressure on primary timber species like; teak, sheesham, sal, deodar etc. and their cost is also high. In this context utilization of secondary species play a very important role. The main drawback with these secondary lesser known timber species are their durability during use for different applications. These species are more biodegradable. Therefore, protection of wood and wood products is required during manufacturing, storage, transportation and also in service [1]. Biological degradation may be due to various causes like bacteria, mold and stain, decay fungi, insects, and marine borers. Mold and stain fungus make the wood more absorptive, which makes the wood more susceptible to moisture and decay fungi. These conventional wood preservatives, although found to be very effective against wood destroying organisms but are said to cause environmental pollution and a few of them are hazardous to animals and human beings etc [2]. Certain wood preservatives have been banned or limited for some applications such as chromated

copper arsenate (CCA) in some European countries, the United States and Japan [3]. Plants are known to be store house of various biological active compounds possessing toxicity against microbes and insects. Therefore, much emphasis is now towards the development of ecofriendly formulations from natural resources such as plants. In recent years, treatment of wood with vegetable based extracts is being given more emphasis so as to develop ecofriendly preservatives [4]. The use of biological means have greater advantages over the use of chemicals for degradation because biotechnologically synthesized products are less toxic and environmental friendly [5]. Therefore, the plant extracts can offer substantial advantages for wood protection, providing decay resistance against fungi at low cost, low mammalian toxicity, ease of handling and treatment [6].

Material and Methods: The present investigation was carried out during the years 2014-2017 in the Laboratory of Department of Forest Products, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh). To study the effect of plant extracts on dimensional stability selected the two plant species viz. *Acorus calamus* L. and *Parthenium hysterophorus* L. The rhizomes of *A. calamus* and aerial parts of *P. hysterophorus* L. were collected from the university campus. For extraction. Two different solvents methanol and petroleum ether were used. The wood samples size, 5cm x 2.5cm x 2.5cm +0.25cm x 0.15cm x 0.15cm (longitudinal x radial x tangential) were used for the investigation. The Six different concentrations ($T_1$0.25%), $T_2$0.50%, T31.00%, $T_4$1.5%, T_5 2.00% T_6 Control) of petroleum ether and methanol extract solutions (w/v) of both species were used for the dip treatment (72 hours), the samples meant for control were dipped in 5 per cent methanol solution prepared with distilled water. After dip treatment, the specimens were first dried in air and then dried at 105$\pm$2°C to constant weights then wood samples treated with fungus culture. Fungal Colonization observations were recorded visually on the comparative growth of the fungus on the treated wood samples. There were three replicates for every treatment in each species and data were analyzed by using the three factorial complete randomized block design (CRD). Observations were recorded visually on the comparative growth of the fungus on the treated wood samples. The following scale was used

Numerical ratings	Description
0	0% area cover
1	1-25% area cover
2	26-50% area cover
3	51-75% area cover
4	>76% area cover

Per cent fungus colonization index was calculated according to [7].

$$\text{Per cent Index} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings x Maximum grade}} \times 100$$

Per cent fungus growth inhibition was calculated by using following formula suggested by [8].

$$I = \frac{C-T}{C} \times 100$$

Where,

I = Per cent fungus growth inhibition
C = Per cent fungus colonization in control
T = Per cent fungus colonization in treatment

Results and discussion

Fungal Colonization: The data obtained for effect of plant extracts treated and untreated wood samples on *T. versicolor* (White rot) presented in table 1. The graphical representation variation in effect of plant extracts on *T. versicolor* (White rot) fungal colonization of treated and untreated wood samples of *Pinus roxburghii* present at figure 1. The data were found significant differences in fungal colonization on treated and untreated woods samples. Fungal colonization data for concentration, plant extract and different solvents for extraction showed significant differences at 5 per cent level of significance. Among concentration, the maximum colonization (100%) was recorded for the control and minimum was noticed in 2.00% concentration (12.86%). Among the extract, the maximum colonization (59.04%) noticed for *A. calamus* treated samples and minimum (53.03%) was found in *P. hysterophorus* treated wood samples. Among the solvents for extraction, the maximum colonization (58.69%) for wood samples treated with petroleum ether extract and minimum value (53.38) was noticed in methanol treated wood samples.

The interaction between extracts and concentrations, concentrations and solvents for extraction and extracts and solvents for extraction were also found to be statistically significant. Interaction between extracts and concentrations, the maximum value of 100% was found in *A calamus* (E_1) extract treated wood samples at control sample and the minimum value (11.10%) was recorded in *P. hysterophorus* L. (E_2) extract treated wood samples at (2.00%) concentration. Interaction between concentrations and solvents for extraction, the highest value (100) was found at control wood samples treated with petroleum ether (S_2) extract and lowest value of (10.12%) was found at (2.00%) concentration wood samples treated with methanol extract (S1). Interaction between plant extracts and solvents for extraction, the highest value of (62.45%) was recorded with *A. calamus* L. in Petroleum ether extract (E_1S_2) and minimum value of (51.13%) was observed with *P. hysterophorus* L. in methanol extract (E_2S_1). The second order interactions between concentrations, extracts and solvents for extraction were found to be significant at 5 per cent level significance. The maximum value of 100% was recorded for $T_6xE_1xS_1$ and minimum value of (9.20%) was found in $T_5xE_2xS_1$.

The data obtained for effect of plant extracts and chromium treated wood samples on *T. versicolor* (White rot) are presented in table 2. The graphical representation variation in effect of plant extracts and chromium on *T. versicolor* (White rot) fungal colonization of treated and untreated wood samples of *Pinus roxburghii* present at figure 2. The data were pertaining significant differences in fugal colonization on treated and untreated woods samples. Fungal colonization data for concentration, plant extract and different solvents for extraction showed significant differences at 5 per cent level of significance. Among concentration, the maximum colonization (76.47%) was recorded for the control wood samples and minimum was noticed in 2.00% concentration (10.31%). Among the extract, the maximum colonization (49.31%) noticed for *A. calamus* treated samples and minimum (46.84%) was found in *P. hysterophorus* treated wood samples. Among the solvents for extraction, the maximum colonization (48.84%) for wood samples treated with petroleum ether. and minimum (47.31%) was noticed in methanol treated wood samples.

The interaction between extracts and concentrations, concentrations and solvents for extraction and extracts and solvents for extraction were found to be statistically significant. Interaction between extracts and concentrations, the maximum value of 77.60% was found in *A calamus* (E_1) extract treated wood samples at control sample and the minimum value (10.13%) was recorded in *P. hysterophorus* L. (E_2) extract treated wood samples at (2.00%) concentration. Interaction between concentrations and solvents for extraction, the highest value (76.48) was obtained at control wood samples treated with petroleum ether (S_2) extract and lowest value of (8.95%) was found at (2.00%) concentration wood samples treated with methanol extract (S_1). Interaction between plant extracts and solvents for extraction, the highest value of (49.34%) was recorded with *A. calamus* L. in methanol extract (E_1S_1) and minimum value of (45.29%) was observed with *P. hyterophorus* L. in methanol extract (E_2S_1). The second order interactions between concentrations, extracts and solvents for extraction were also found to be significant at 5 per cent level significance. The maximum value of (78.35%) was recorded for $T_6xE_1xS_1$ and minimum value of (8.85%) was found in $T_5xE_2xS_1$. The fungus has colonized more in plant extracts treated samples as compared to the plant extracts and Chromium treated samples. Similar results were reported by [9] reported the no fungal growth fungus on the wood surface treated with methanol extract of *Maclura pomifera* bark and all the other treatments have shown the growth of *Fusarium culmorum* on different extracts treated wood samples. The wood samples treated with Paraloid B-72 and methanol extracts of *M. pomifera* at superior concentration (10%) have shown no observable growth of *F. culmorum* on the wood surface. [10]] studied the seven Amazonian woods extracts, *Bagassa guianensis, Manilkara huberi, Sextonia rubra, Vouacapoua americana, Andira surinamensis, Handroanthus serratifolius, and Qualea rosea*) six wood extracts had shown efficacy against soft-rot fungi. In particular, the preservation efficacies of *B. guianensis, H. serratifolius,* and *S. rubra* extracts were highly significant up to retention levels of 23, 25, and 12 kg m3, respectively. Three extracts (*A. surinamensis, H. serratifolius*, and *Q. rosea*) were then tested against *Gloeophyllum trabeum* (brown rot) and *Trametes versicolor* (white rot) *H. serratifolius* wood extract was found very efficient at protecting *P. sylvestris* samples at 5.1 kg m3 against the brown rot [11]. also evaluated the potential of neem (*Azadirachta indica*) seed oil as bio-preservative against termite attack on *Aningeria robusta* wood.

Fungal inhibition

The data obtained for effect of plant extracts on *T. versicolor* (White rot) fungal inhibition of treated wood samples are presented in table 3. The graphical representation of variation in effect of plant extracts on *T. versicolor* (White rot) fungal inhibition of treated wood samples of *Pinus roxburghii* are present at figure 3. The data were found significant differences in fugal inhibition on treated and untreated woods samples. Fungal inhibition data for concentrations, plant extracts and different solvents for extraction found significant differences at 5 per cent level of significance. Among concentration, the maximum inhibition (87.19%) was noticed for the (2.00%) concentration and minimum was found in 0.25% concentration (21.18%). Among the extract, the maximum inhibition (56.37%) found for *P. hysterophorus* treated samples and minimum (49.27%) was found in *A. calamus* treated wood samples. Among the solvents for extraction, the maximum inhibition (56.06%) for wood samples treated with methanol extract and minimum value (49.57%) was found in petroleum ether treated wood samples.

The interaction between extracts and concentrations, concentrations and solvents for extraction and extracts and solvents for extraction were found to be statistically significant. Interaction between extracts and concentrations, the maximum value (88.90%) was found in *P. hysterophorus* (E_2) extract treated wood samples at (2.00%) and the minimum value (16.82%) was recorded in *A. calamus* L. (E_1) extract treated wood samples at (0.25%) concentration. Interaction between concentrations and solvents for extraction, the highest value (89.98) was found at 2.00% concentration with methanol (S_1) extract and minimum value of (18.44%) was noticed at (0.25%) concentration wood samples treated with petroleum ether (S_1). Interaction between plant extracts and solvents for extraction, the highest value of (58.65%) was recorded with *P. hysterophorus* in methanol extract (E_2S_1) and minimum value of (45.07%) was observed with *A. calamus*. in petroleum ether (E_1S_2). The second order interactions between concentrations, extracts and solvents for extraction were also found to be significant at 5 per cent level significance. The maximum value of (90.80%) was recorded for $T_5xE_2xS_1$ and minimum value of (14.41%) was found in $T_1xE_1xS_2$. The data obtained for effect of plant extracts and chromium on *T. versicolor* (White rot) fungal inhibition of treated wood samples presented in table 4. The graphical representation on variation in effect of plant extracts and chromium on *T. versicolor* (White rot) fungal inhibition of treated wood samples of *Pinus roxburghii* Sargent present on figure 4. The data were noticed significant differences in fugal inhibition on treated and untreated woods samples. Fungal inhibition data for concentration, plant extract and different solvents for extraction found significant differences at 5 per cent level of significance. Among concentration, the maximum inhibition (90.41%) was noticed for the (2.00%) concentration and minimum was found in 0.25% concentration (10.44%). Among the plant extract, the maximum inhibition (49.44%) found for *P. hysterophorus* treated samples and minimum (43.75%) was found in *A.*

calamus treated wood samples. Among the solvents for extraction, the maximum inhibition (49.81%) for wood samples treated with methanol extract and minimum value (43.37%) was found in petroleum ether treated wood samples. The interaction plant extracts and solvents for extraction were found to be statistically significant and interaction between plant extracts and concentrations and concentrations and solvents used for extraction were found non-significant. Interaction between plant extracts and solvents for extraction, the highest value of (55.19%) was recorded with *P. hysterophorus* in methanol extract (E_2S_1) and minimum value of (43.06%) was observed with *A. calamus*. in petroleum ether (E_1S_2). Interaction between plant extracts and concentrations and interaction between concentrations and solvents for extraction value range occur between 9.51 to 94.37 and 8.49 to 69.08 respectively. The second order interactions between concentrations, extracts and solvents for extraction were also found non- significant data range from 7.92 to 94.37 [12]. reported similar result natural plant extracts, such as essential oils, for use on wood. Seven essential oils were evaluated for their ability to inhibit growth of *Aspergillus niger, Trichoderma* viride, and *Penicillium chrysogenum* on southern yellow pine (SYP) stakes that were either dip treated or exposed to vapours of the test oils. Thyme and Egyptian geranium oil inhibited growth of all test fungi for 20 weeks [13]. investigated the fungitoxic activities of neem leaves extractives against wood decay fungi [14]. studied Port-Orford cedar (Chamaecyparis lawsoniana), Alaska yellow cedar (*Chamaecyparis nootkatensis*), and Eastern red cedar (*Juniperus virginiana*) these were substituted to supercritical fluid extraction with CO_2 (SCC) and Soxhlet extracted (SE) with hexane. The oils were evaluated against two common wood decay fungi, brown-rot fungus (*Gloeophyllum trabeum*) and white-rot fungus (*Trametes versicolor*) [15]. studied the eco-friendly management of timber degrading fungi is tried by using plant extracts, oils and gels. Nagadesi and Arya (2016) also reported the eco-friendly management of timber degrading fungi is tried by using plant extracts, oils and gels. In most of the fungi 25% methanolic extract was more effective than 5 and 10% concentrations. *Lenzites sterioides* was completely inhibited by 5% leaf extract of *P. Juliflora* and 10% leaf extracts of *Prosopis, Cymbopogon* and Datura at 25% concentration. Oils and gels of Cymbopogon citrates, *Anacardium occidentale* L., *Gossypium barbadensis* L., *Linum usitatissimum* L., *Aloe vera* L., and *Aloe ferox* Mill. were used to control the timber degrading fungi.

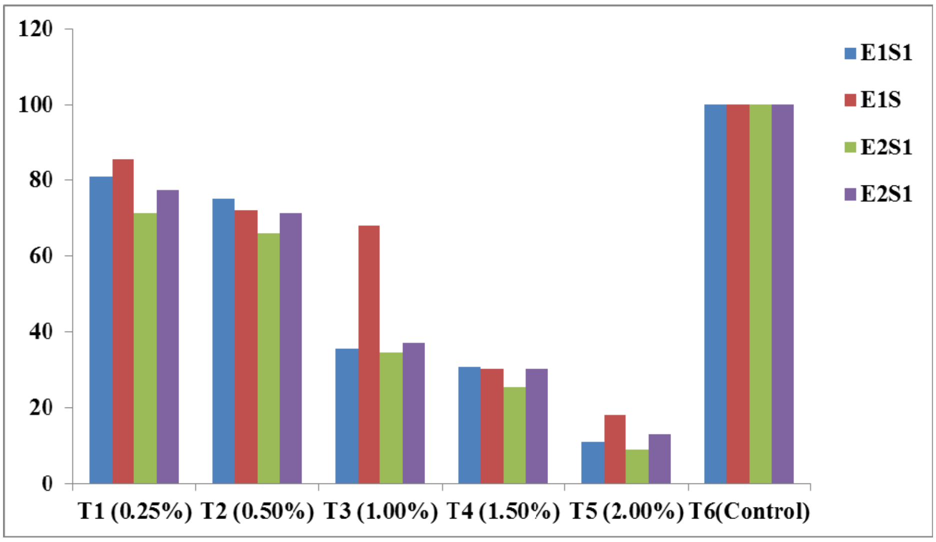

Fig 1: Variation in effect of plant extracts on *Trametes versicolor* (White rot) fungal colonization of treated wood samples of Pinus *roxburghii* Sargent

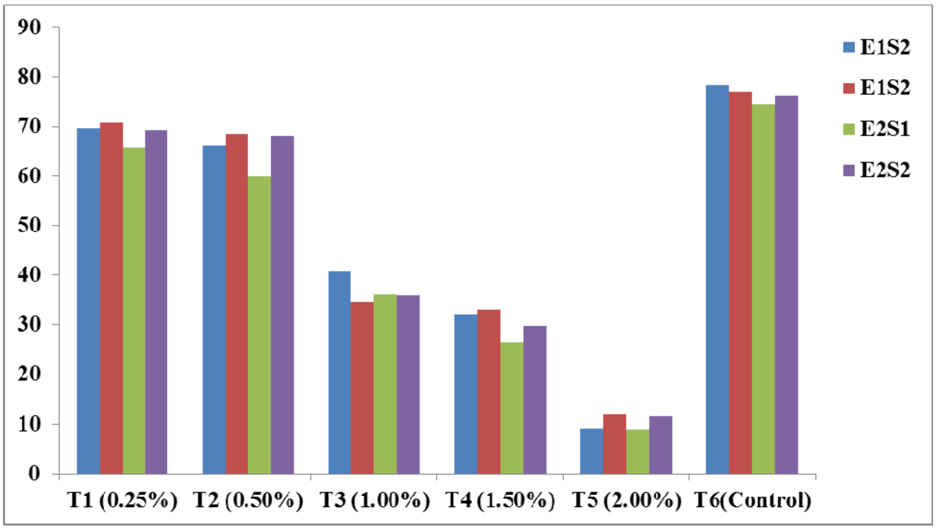

Fig 2: Variation in effect of plant extracts and chromium on *Trametes versicolor* (White rot) fungal colonization of treated wood samples of *Pinus roxburghii* Sargent.

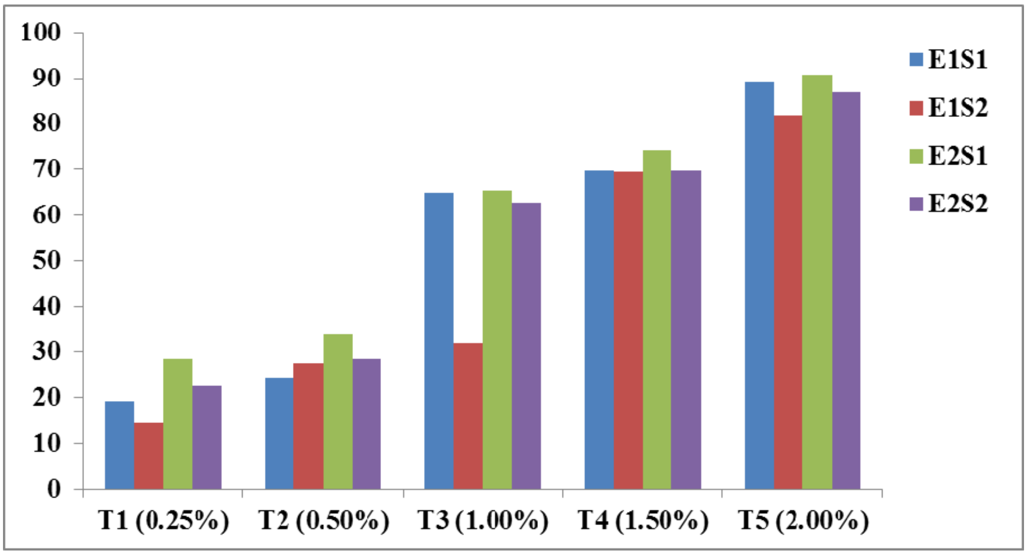

Fig 3: Variation in effect of plant extracts on *Trametes versicolor* (White rot) fungal inhibition of treated wood samples of *Pinus roxburghii* Sargent.

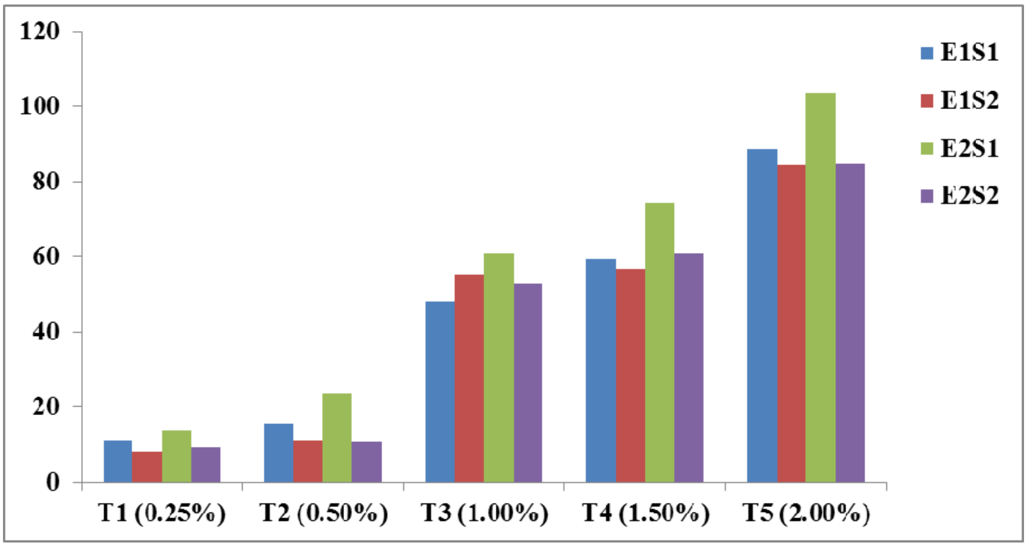

Fig 4: Variation in effect of plant extracts and chromium on *Trametes versicolor* (White rot) fungal inhibition of treated wood samples of *Pinus roxburghii* Sargent.

Table 1: Effect of plant extracts on *Trametes versicolor* (White rot) fungal colonization of treated wood samples of *Pinus roxburghii* Sargent

Concentrations (T)	Per cent Fungal Colonization								
	Plant extracts (E)						Solvents for extraction (S)		Mean
	Acorus calamus L.(E_1)			*Parthenium hysterophorus* L. (E_2)			Methanol (S_1)	Petroleum ether (S_2)	
	Methanol (S_1)	Petroleum ether (S_2)	Mean	Methanol (S_1)	Petroleum ether (S_2)	Mean			
T_1 (0.25%)	81.01	85.60	83.30	71.38	77.54	74.46	76.19	81.57	78.88
T_2 (0.50%)	75.26	72.30	73.78	65.89	71.56	68.72	70.57	71.93	71.25
T_3 (1.00%)	35.70	68.14	51.92	34.63	37.16	35.89	35.16	52.65	43.90
T_4 (1.50%)	30.83	30.45	30.64	25.68	30.33	28.00	28.25	30.39	29.32
T_5 (2.00%)	11.05	18.20	14.62	9.20	13.01	11.10	10.12	15.60	12.86
T_6(Control)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean	55.64	62.45	59.04	51.13	54.93	53.03	53.38	58.69	

$CD_{0.05}$

Concentration (T)	0.53
Extract (E)	0.31
Solvent (S)	0.31
T*E	0.76
T*S	0.76
E*S	0.44
T*E*S	1.07

Table 2: Effect of plant extracts and chromium on *Trametes versicolor* (White rot) fungal colonization of treated wood samples of *Pinus roxburghii* Sargent

Concentrations (T)	Per cent Fungal Colonization								
	Plant extracts (E)						Solvents for extraction (S)		Mean
	Acorus calamus L.(E_1)			*Parthenium hysterophorus* L. (E_2)			Methanol (S_1)	Petroleum ether (S_2)	
	Methanol (S_1)	Petroleum ether (S_2)	Mean	Methanol (S_1)	Petroleum ether (S_2)	Mean			
T_1 (0.25%)	69.66	70.77	70.21	65.83	69.21	67.52	67.75	69.99	68.87
T_2 (0.50%)	66.20	68.35	67.27	59.92	67.96	63.94	63.06	68.16	65.61
T_3 (1.00%)	40.80	34.63	37.71	36.08	35.83	35.96	38.44	35.23	36.83
T_4 (1.50%)	31.96	33.13	32.55	26.46	29.86	28.16	29.21	31.49	30.35
T_5 (2.00%)	9.05	11.96	10.50	8.85	11.41	10.13	8.95	11.68	10.31
T_6(Control)	78.35	76.86	77.60	74.58	76.10	75.34	76.47	76.48	76.47
Mean	49.34	49.28	49.31	45.29	48.39	46.84	47.31	48.84	

CD0.05

Concentration (T)	0.44
Extract (E)	0.26
Solvent (S)	0.26
T*E	0.63
T*S	0.63
E*S	0.36
T*E*S	0.89

Table 3: Effect of plant extracts on *Trametes versicolor* (White rot) fungal inhibition of treated wood samples of *Pinus roxburghii* Sargent

Concentrations (T)	Per cent Fungal Inhibition								
	Plant extracts (E)						Solvents for extraction (S)		Mean
	Acorus calamus L.(E_1)			*Parthenium hysterophorus* L. (E_2)			Methanol (S_1)	Petroleum ether (S_2)	
	Methanol (S_1)	Petroleum ether (S_2)	Mean	Methanol (S_1)	Petroleum ether (S_2)	Mean			
T_1 (0.25%)	19.24	14.41	16.82	28.63	22.47	25.55	23.93	18.44	21.18
T_2 (0.50%)	24.48	27.71	26.09	34.12	28.44	31.28	29.30	28.07	28.69
T_3 (1.00%)	64.86	31.87	48.36	65.38	62.85	64.11	65.12	47.36	56.24
T_4 (1.50%)	69.59	69.56	69.57	74.33	69.67	72.00	71.96	69.61	70.79
T_5 (2.00%)	89.16	81.80	85.48	90.80	87.00	88.90	89.98	84.40	87.19
Mean	53.47	45.07	49.27	58.65	54.08	56.37	56.06	49.57	52.82

(*) Per cent growth inhibition over control

CD	0.05
Concentration (T)	0.57
Extract (E)	0.36
Solvent (S)	0.36
T*E	0.81
T*S	0.81
E*S	0.51
T*E*S	1.14

Table 4: Effect of plant extracts and chromium on *Trametes versicolor* (White rot) fungal inhibition of treated wood samples of *Pinus roxburghii* Sargent

Concentrations (T)	Per cent Fungal Inhibition								
	Plant extracts (E)						Solvents for extraction (S)		Mean
	Acorus calamus L.(S_1)			*Parthenium hysterophorus* L. (S_2)			Methanol (S_1)	Petroleum ether (S_2)	
	Methanol (S_1)	Petroleum ether (S_2)	Mean	Methanol (S_1)	Petroleum ether (S_2)	Mean			
T_1 (0.25%)	11.09	7.92	9.51	13.70	9.06	11.38	12.40	8.49	10.44
T_2 (0.50%)	15.51	11.06	13.29	23.48	10.69	17.09	19.50	10.88	15.19
T_3 (1.00%)	47.93	54.95	51.44	60.67	52.92	56.79	54.30	53.93	54.12
T_4 (1.50%)	59.21	56.90	58.05	74.37	60.77	67.57	66.79	58.83	62.81
T_5 (2.00%)	88.45	84.45	86.45	103.72	85.01	94.37	96.08	84.73	90.41
Mean	44.44	43.06	43.75	55.19	43.69	49.44	49.81	43.37	46.59

(*) Per cent growth inhibition over control

CD0.05

Concentration (T)	3.97
Extract (E)	2.51
Solvent (S)	2.51
T*E	NS
T*S	NS
E*S	3.55
T*E*S	NS

Conclusion

It is concluded in the present investigation the fungal colonization maximum was observed in untreated wood samples and minimum was noticed in 2.00% concentration treated wood samples. Growth of fungus on wood samples were decreases with the increasing the plant extract concentrations. The fungal inhibition highest found at 2.00% concentration and lowest was recorded for the untreated wood samples. However, both plant extracts were found effective against the biodegraded agencies, but *Parthenium hysterophorus* extract was more effective as compare to *Acorus calamus* extract.

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