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Resistance of Commercial and Non-commercial Woods against *Heterotermes indicola* Wasmann (Blattodea: Rhinotermitidae) in Laboratory and Field Conditions

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NAQ and KZR conceived, designed and supervised work. MA collected field data and performed laboratory bioassay. IM and MA statistically analyzed the data. MA and NAQ wrote the article.

Key words
***Heterotermes indicola*, Wood resistance, Feeding preference, Visual rating.**

ABSTRACT

The lower subterranean termite*, Heterotermes indicola* Wasmann is among the most devastating species causing significant loss annually in South Asia. Feeding deterrence of *H. indicola* among 10 different woods species were evaluated in choice and no-choice assays conducted under laboratory and field conditions. Visual rating according to (AWPA, 1997) scale, mass loss, wood consumption per termite and mortality rate were determined to evaluate resistance of wood against *H. indicola*. *Populus euramericana* and *Dalbergia sissoo* were observed most palatable and resistant wood species, respectively, in no-choice laboratory and field trials. Five different wooden pairs were evaluated in choice bioassays in laboratory and field conditions. In force feeding field bioassays, least mass loss was recorded on *D. sissoo* (0.19±0.07g) and maximum on *P. euramericana* (1.50.1g). Under choice laboratory trials, maximum mass loss difference was recorded on pair of *P. euramericana* and *D. sissoo* (0.89±0.05 and 0.14±0.01g), whereas, minimum mass loss difference was observed on pair of *B. variegata* and *A. lebbeck* (0.48±0.02 and 0.43±0.04g). Under choice field conditions, *D. sissoo* and *S. cumini* were found most resistive, whereas, *P. euramericana* and *B. monosperma* were most palatable for *H. indicola.* Feeding tendency in descending order of resistance based on mean mass loss as a quantitative parameter was recorded as: *D. sissoo*, *S. cumini*, *P. roxburghii*, *J. mimosifolia*, *B. variegata*, *M. indica*, *A. lebbeck*, *A. indica*, *B. monosperma*, *P. euramericana*.

INTRODUCTION

Termites feed on a large variety of wood in the world. However, there is diversity in wood species having persistence or antifeeding chemicals against insects. Certain wood species are resistant and least preferred by termites but palatability is strongly accepted parameter to determine consumption rate for natural selection of wood species by termites (Rasib *et al*., 2014). Currently termites prevail in tropical, subtropical and temperate regions of world (Wood, 1978). About 2800 species and 270 genera are known which represent 70% of total described species (Abe *et al*., 2000; Bignell *et al*., 2004). Akhtar (1983) reported that out of 50 termite species found in Pakistan, only 11 are of strong economic important pest *e.g. Heterotermes indicola, Coptotermes heimi*, *Microtermes obesi* and *Microtermes unicolor* from Punjab and NWFP (now KPK) and *Anacanthotermes vegans,* most devastating termite in Baluchistan.

Lower termites are considered as invasive pests which cause tremendous loss to wooden structures in commercial and house buildings, agriculture crops and forestry crops. An estimated cost of wooden loss and their maintenance approximately $3000 million annually in United States (Pimentel *et al*., 2005). Natural resistance of local timber against different invasive termite species of Pakistan has been reported by various entomologists (Akhtar and Ali, 1979; Manzoor *et al*., 2009; Rasib and Ashraf, 2014). However, the natural resistance of common wood species in Pakistan still has not received an adequate consideration and has not applied in field, whereas, in different ecological zones of world most resistant wood species taken as a standard of knowledge of termites wood resistant (Gazal *et al*., 2010). *Heterotermes indicola* is the most

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notorious subterranean, wood-destroying lower termite in Pakistan. It attacks on a wide range of trees including: Mulberry (*Morus alba*), Akk (*Calotropis procera*), Lokat (*Erioborrya japonica), Dalbergia sissoo*, *Acacia* spp., *Populus euramericana*, *Melia azedarach* and dead woods (Chaudhry and Ahmad, 1972). Around the world natural durability of wood is classified in different ways. The heartwood of many timber species is to some degree resistant to biological breakdown. This is termed natural durability and may refer to a wood's resistance to decay, marine borers, termites or other insects (Stirling, 2009). In entire world there is a traditional knowledge on the natural durability of local woods (Willeitner and Peek, 1997).

The present research was aimed to evaluate resistance of ten important wood species against subterranean termites *H. indicola* (both in laboratory and field trials). The results of this research will remind to alert on the use of wood species in household and at commercial level.

MATERIALS AND METHODS

Termite collection

For laboratory bioassays termites were collected by baiting technology from the infested vegetation of F. C. College Lahore, Pakistan. The baits were brought in laboratory after fifteen days; healthy and active workers of *H. indicola* were separated in petri dishes and kept at 25^{0}C for experimental use.

Wood species

Ten wood species; *Azadirachta indica* (Neem), *Pinus roxberghii* (Chir), *Populus euramericana* (Popular), *Dalbergia sissoo* (Sheesham), *Albizia lebbeck* (Shreen), *Butea monosperma* (Dhaak), *Bauhinia variegata* (Kachnar), *Syzygium cumini* (Jamin), *Magnifera indica* (Mango) and *Jacaranda mimosifolia* (Gul-e-neelum) were purchased from the commercial timber market for experimental use . Specific gravity of each wood species was calculated using formula (Simpson, 1993).

$$G_b = \frac{W_d/V_d}{p_w}$$

Where, G_b is basic specific gravity of wood, W_d is the dry weight of wooden blocks (g), V_d is volume of dry-wood volume (m^3) and p_w is density of water (1000kg/m^3).

Test site

The field test was conducted at active nests in infested vegetation of B.R.B canal of Lahore and F.C. College botanical garden site.

No-choice field trials

Test blocks of each wood species measuring (2.5×2.5× 1cm), were tied up into a bundle with a wire. The bundles of all wood species were connected with each other to make long chain. Likewise, three replicates were prepared and each replicate was whirled around infested tree at two described sites (F. C. College, Lahore and B.R.B canal vegetation, Lahore). All replicates were treated with sugarcane juice as attractant for termites and also to keep moisture. At the end of 30 days experiment, wood samples were brought back to laboratory, cleaned, oven dried and weighed to determine the amount of wood consumed.

Choice field trails

A series of paired choice feeding tests were conducted to compare resistance between wood species pairs against *H. indicola*; *P. euramericana* vs *D. sissoo* (PE/DS), *P. roxburghii* vs *A. indica* (PR/AI), *B. variegata* vs *A. lebbeck* (BV/AL), *S. cumini* vs *B. monosperma* (SC/BM) and *M. indica* vs *J. mimosifolia* (MI/JM). Test blocks of 10 wood species measuring (2.5× 2.5× 1cm^3), oven dried for 24 h at 60°C and weighed prior to experiment. Three replicates (n=3) of each group were prepared. All wooden blocks were tied up by copper wire into a bundle and whirled around tree trunks. At the end of 30 days of experiment, wood samples were brought back to laboratory, cleaned, oven dried and weighed to determine the amount consumed.

No-choice laboratory trails

Wooden blocks of ten tested woods measuring (2.5×2.5× 1cm^3) were prepared, oven dried at 60°C for 24 h and soaked in distilled water for moisture for termites. Only one type of test block was placed in a glass petri dish (9.5 cm in diameter and 2 cm high) and 50 termite workers of *H. indicola* were added in each petri dish. Petri dishes were maintained at 26^{o}C for 14 days in dark. Each experiment was designed in triplicate (n=3). At the end of the test period, percentage mortality of *H. indicola* was calculated against each wood species according to the formula:

$$\text{Mortality \%} = \frac{\text{ODP}}{\text{TP}} \times 100$$

Where, ODP is observed dead population of workers and TP is total population of workers

The amount of wood consumed was calculated after 14 days using formula:

$$\%\text{Weight loss} = \frac{W_b - W_a}{W_b} \times 100$$

Where, W_b is wooden block's weight before experiment and W_a is wooden block's weight after experiment (Sotannde *et al*., 2011).

Wood consumption per individual (g) was also calculated by the formula:

$$W_b = W_a / N$$

Where, N is number of initial workers.

Choice laboratory trails

Wooden blocks were prepared in the same way as in no choice tests, except that wooden blocks of two different plant species were offered as food source in one petridish in the same experiment. Three replicates (n=3) of each experiment were maintained.

Visual rating of wood species

In both laboratory and field trials, tested wood specimens were assessed according to the (AWPA, 1997). Visual rating of the test blocks using the scale of 10 (sound, surface nibbles permitted), 9 (light attack), 7 (moderate attack), 4 (heavy attack), or 0 (no attack). After 14 (laboratory) and 30 days (field trials), the assessment was carried out on the wood specimens covering percentage of wood weight loss, percentage of survival species and degree of termite attack.

Statistical analysis

Data for wood consumption obtained from no-choice bioassays were subjected to analysis of variance (ANOVA) and significant difference among mean values was separated by Tukey HSD test at 5 percent level. Furthermore, Pearson correlation was applied to find out relationship between specific gravity and wood consumption (Altman and Bland, 1983). Results obtained from choice bioassays were analyzed for comparison of wood consumption among wooden pairs using paired *t*-test (SPSS version 19).

RESULTS

Visual rating

Results revealed that the woods of *D. sissoo* and *S. cumini* were very resistant (VR), woods of *P. roxburghii* and *J. mimosifolia* were resistant (R), woods of *A. indica, A. lebbeck, B. variegata* and *M. indica* were moderately resistant (MR) whereas, *P. euramericana* and *B. monosperma* were susceptible (S) during choice and no-choice in both laboratory and field conditions.

No-choice feeding field bioassays

Results of one-way ANOVA (unstacked) showed that the mean wood consumption was significantly (DF= 3; F= 112.06; P=0.000) different of all wood species used against *H. indicola*. The assessment of *H. indicola* inflicted mass loss (g) among wooden blocks of 10 wooden species showed that the wood of *D. sissoo* was highly resistant and mean mass loss by blocks of *D. sissoo* was significantly less than other wood species, whereas mass loss 1.50±0.10g in *P. euramericana* blocks after 30 days of exposure was found maximum. *S. cumini* ranked second most resistant after *D. sissoo*. Table I shows other most palatable wood was *B. monosperma* after *P. euramericana.* Visual rating of termite infested wooden blocks was the most preferred wood. Woods were arranged in the following descending order of resistance against *H. indicola* DS> SC>PR> JM> BV> MI> AI> AL> BM PE.

Table I.- Results of visual rating and mean mass loss (g) of 10 wood species exposed to *H. indicola* for 30 days in no-choice field assays.

Wood species	Visual rating	Mass loss g (Mean±SD)
Populus euramericana (PE)	4 (S)	1.50±0.10^{a}
Azadirachta indica (AI)	7 (MR)	0.61±0.15^{b}
Pinus roxberghii (PR)	9 (R)	0.24±0.52^{c}
Dalbergia sissoo (DS)	10 (VR)	0.19±0.07^{cd}
Albizia lebbeck (AL)	6 (MR)	0.75±0.23^{b}
Butea monosperma (BM)	4 (S)	0.99±0.05^{ae}
Bauhinia variegata (BV)	7 (MR)	0.54±0.16^{f}
Syzygium cumini (SC)	10 (VR)	0.22±0.01^{cdg}
Mangifera indica (MI)	8 (MR)	0.51±0.03^{fh}
Jacaranda mimosifolia (JM)	8 (MR)	0.47±0.05^{fh}

Tukey, HSD tests showed the means followed by different letters within column indicate that means are significantly different (P<0.05). S, susceptible; MR, moderate resistant; R, resistant; VR, very resistant.

Choice feeding field bioassays

Among the wooden blocks offered in pair of two species to *H. indicola* showed the significant (P=0.004) feeding (1.95±0.01g) on *P. euramericana* and minimum (0.12±0.23g) on *D. sissoo* (Table II). In the field choice feeding bioassay the woods were arranged in the following descending order of resistance against *H. indicola* DS> PR> SC> JM> BV> MI> AL> AI> BM> PE. Results of choice feeding trials also indicated that *H. indicola* like other termite species capable of recognizing palatable and avoiding from these woods blocks which have natural deterrent properties.

Table II.- Comparative results of visual rating and mean mass loss (g) of five wood pairs during (choice feeding) bioassay against *H. indicola* after the 30 days exposure in field conditions.

Wood species	Mean visual rating		Mean mass loss g (Mean±SD)		Probability	t-value	95% CI
	W1	W2	W1	W2			
DS/PD	10(VR)	4 (S)	0.12±0.03	1.95±0.11	0.004*	-16.17	(-2.347, -1.360)
PR/AI	9(R)	7 (MR)	0.30±0.06	0.76±0.14	0.010*	-10.59	(-0.695, -0.293)
BV/AL	7 (MR)	7 (MR)	0.63±0.01	0.75±0.14	0.264	-1.11	(-0.585, 0.345)
BM/SC	4 (S)	10(VR)	1.90±0.02	0.32±0.02	0.009*	-12.65	(-1.678, -0.333)
MI/JM	7 (MR)	9 (R)	0.67±0.02	0.44±0.06	0.383	1.54	(-0.402, -0.850)

Difference in mass loss for each pair of wooden block indicated by P<0.05* are highly significant (paired comparison t-test). W1, Wood1; W2, Wood2 (For each pair). For abbreviations, see Table I.

Table III.- Results of mean visual rate, mean mass loss (g), consumption% (Correlation) and specific gravity of 10 wood species exposed to *H. indicola* for 14 days in no-choice laboratory assays.

Wood species	Visual Rating	Mass loss (g) (g/termite/day)	Consumption% (Correlation)	Specific gravity
Populus euramericana (PD)	4 (S)	0.99±0.34^{a} (0.019)	48.36 (*r*= 1.00)	0.121
Azadirachta indica (AI)	7 (MR)	0.45±0.05^{b} (0.009)	12.64 (*r*= 0.88)	0.417
Pinus roxberghii (PR)	9(R)	0.22±0.04^{c}(0.004)	07.45 (*r*= 0.85)	0.489
Dalbergia sissoo (DS)	10(VR)	0.12±0.03^{d} (0.002)	02.04 (*r*= -0.92)	0.572
Albizia lebbeck (AL)	7 (MR)	0.38±0.12^{e} (0.007)	10.50 (*r*= 0.98)	0.539
Butea monosperma (BM)	4(S)	0.76±0.09^{af} (0.015)	34.68 (*r*=1.00)	0.231
Bauhinia variegata (BV)	7(MR)	0.32±0.05^{eg} (0.006)	08.44 (*r*=0.95)	0.588
Syzygium cumini (SC)	10(R)	0.12±0.01^{dh} (0.002)	02.80 (*r*= -0.98)	0.754
Magnifera indica (MI)	7(MR)	0.45±0.06^{bi} (0.009)	12.00 (*r*= 0.99)	0.404
Jacaranda mimosifolia (JM)	9(R)	0.23±0.02^{bj} (0.004)	08.90 (*r*=0.85)	0.622

Tukey, HSD tests showed the means followed by different letters within column indicate that means are significantly different (P<0.05). Positive and sig. P<0.05 (2-tailed) relationship between consumption % and specific gravity by Pearson correlation. For abbreviations see, Table I.

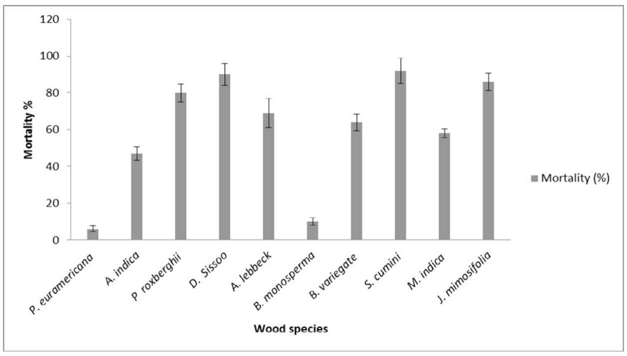

Fig. 1. Mean mortality (%) of *H. indicola* workers in no-choice laboratory feeding trials on 10 different wood species blocks after 14 days of continuous exposure.

No-choice feeding laboratory assay

Regarding the mean wood mass laboratory trials, results indicated that highest mean mass loss was 0.99±0.34 g for *P. euramericana* and lowest mean mass loss for *D. sissoo* was 0.12±0.03 g that were statistically significant (DF=3; F= 72.80; P<0.05). From the Figure 1, it is also evident that the termite percentage mortalities for *P. euramericana, A. indica, P. roxburghii, D. sissoo, A. lebbeck, B. monosperma, B. variegata, S. cumini, M. indica* and *J. mimosifolia* were 6, 47, 80, 90, 69, 10, 64, 92, 58, and 86%, respectively. It was also concluded that the wood of *D. sissoo* and *S. cumini* were least preferred, whereas, *P. euramericana* and *B. monosperma* were mostpreferred woods for *H. indicola*.

Table IV.- Comparative results of visual rating and mean mass loss (g) of ten woods species offered in combinations during (choice feeding) bioassay against *H. indicola* after the 14 days in laboratory conditions.

Wood pairs	Visual rating		Mean mass loss (g)		Probability	*t-value*	95% CI
	W1	W2	W1(X±SE)	W2(X±SE)			
PD/DS	10 (VR)	4(S)	0.89±0.05	0.14±0.01	0.001*	32.67	(0.3988, 1.1032)
PR /AI	9 (R)	7(MR)	0.28±0.04	0.54±0.27	0.046	-8.21	(-0.3871, -0.1209)
BV/AL	7(MR)	8(MR)	0.48±0.02	0.43±0.04	0.408	1.04	(-0.1696, 0.2776)
BM/SC	4(S)	10(VR)	0.85±0.09	0.15±0.03	0.005*	9.17	(0.6061, 0.7899)
MI/JM	7(MR)	8(MR)	0.56±0.10	0.28±0.05	0.012	9.21	(0.1497, 0.4123)

Highly significantly difference in mass loss for each pair of wooden block indicated by *P<0.05. (paired comparison *t*-test). For abbreviations see, Table I.

In the laboratory no-choice feeding bioassay the woods are arranged as following descending order of resistance for *H. indicola* DS> SC>PR> JM> BV> MI> ALAI> BM> PE (Table III). Analysis of variance revealed mass loss of different woods is significantly different from one another. The correlation between weight loss of *D. sissoo* and its specific gravity was statistically negative (*r*= -0.92), whereas, the correlation among weight loss of *P. euramericana* and its specific gravity was statistically positive and significant (*r*= 1.00).

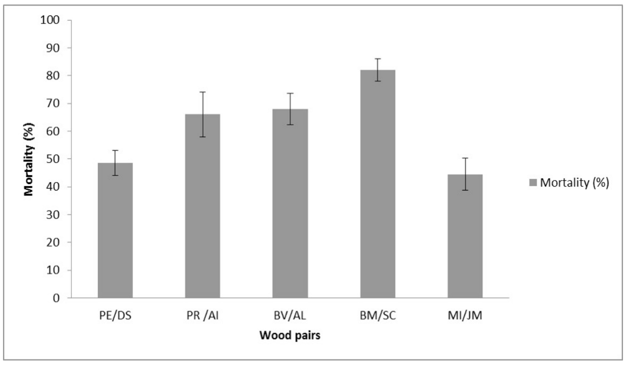

Fig. 2. Mean mortality (%) of *H. indicola* workers in choice laboratory feeding trials on 5 different wooden pairs blocks after 14 days of continuous exposure.

Choice feeding laboratory assay

The results of laboratory choice feeding test against *H. indicola* indicated that the wood of *P. euramericana* was significantly (P=0.000) preferred than wood of *D. sissoo*. The wood of *A. indica* was also preferred more than *P. roxburghii.* A non-significant feeding preference was observed among the pair of *B. variegata* and *A. lebbeck*, while a significant (P=0.005) resistance of *S. cumini* was observed than *B. monosperma.* Furthermore, among the pair of *M. indica* (MI) and *J. mimosifolia* (JM) non-significant (P=0.070) mass loss (g) difference was observed which depict *J. mimosifolia* was more resistant against *H. indicola* than *M. indica* (Table IV). The termite percentage mortality for *P. euramericana* vs *D. sissoo* (PE/DS), *P. roxberghii* vs *A. indica* (PR/AI), *B. variegata* vs *A. lebbeck* (BV/AL), *S. cumini* vs *B. monosperma* (SC/BM) and *M. indica* vs *J. mimosifolia* (MI/JM) were 48.5, 66, 68, 82, 69 and 44.5%, respectively (Fig. 2). It was concluded regarding the mean wood mass, that highest mean mass loss was for *P. euramericana* (0.892±0.257g) and lowest mean mass loss was for *D. sissoo* (0.140±0.010g) (Table IV).

DISCUSSION

Feeding deterrence is a serious indicator of the lifespan of tree species; the main objective of the current study was to illuminate which species of local timber shows natural deterrent against *H. indicola* the most economically important termite species and which woods species were palatable. In our study, the results of wood consumption showed that *D. sissoo*, *S. cumini* and *P. roxburghii* have inherent natural resistance that protects it from *H. indicola* attack. Physical and chemical composition of wood species *e.g.* hardness, bioactive chemical contents, high concentration of lignin present in their bark, sapwood and heartwood may hinder the feeding activity of termite. The organic constituents such as terpenoids, phenol and quinines may vary in the degree of resistance of wood species against termites (Yohannes, 2006). In the present findings choice feeding trials were more successful than no-choice (force feeding) assays for determining wood resistance against termites because in no-choice sources termites were forced to feed whatever source was available for food. It is evaluated in the laboratory choice assays that *H. indicola* could easily differentiate most palatable and resistant wood. Getachew *et al.* (2003) suggested some termites species have a propensity for definite wood preferences, like dry wood termites prefer fast growing tissues of wood, *e.g*. spring wood growth, that produce large cells with thin cell walls and fibers.

In this study, it was also concluded that more durable wood species owe their resistance due to their hardness and extractive which may serve as natural preservatives. *D. sissoo* has high durability (hardness) which helped for its avoidance against *H. indicola* than *P. euramericana;* similarly *S. cumini* may have natural preservatives that showed high resistance than *B. monosperma.* It has been investigated that wood susceptibility increase with different environmental factors such as moisture contents, lignifications attack by fungus and age of wood which determine its compactness (Peacrce, 1997; Qureshi *et al*., 2012a). In this study, wood blocks of *P. euramericana*, *B. monosperma* have highly consumption rate against *H. indicola* compared to all other wood species; on other hand *D. sissoo, S. cumini, P. roxburghii* and *J. mimosifolia* were antifeedant against termite workers.

In present study, a significant correlation was observed between specific gravity, cellulose, density, hardness, total bioactive ingredients in wood and destruction of wood by *H. indicola*. It was found that the increase in density and specific gravity of wood is directly proportional to their resistance level. Similarly, hardness, lignin and other bioactive ingredients also guaranteed higher resistance; whereas cellulose contents increased the feeding preference of *H. indicola*. It was also observed that *D. sissoo* and *S. cumini* were highly resistant to *H. indicola*, which make an agreement between previous (Manzoor *et al*., 2009; Shanbhag and Sunararaj, 2013; Rasib *et al*., 2014) and present study. In no-choice laboratory trial, mass loss of woods by termites varied from 0.004 to 0.019 g/termite/day, such variation in mass loss of wood may be due to their physical and chemical nature. Percentage mortality also revealed that workers of *H indicola* were successfully 50% survived on *P. euramericana* and more than 50% termites were failed to survive on other wood species (Fig. 1). Rasib *et al*. (2014) reported that workers of *O. obesus* were failed to survive during force feeding in 14 days laboratory assays. However our findings approximately resemble with reported results. Many researchers have investigated the termite ecology, termite inflicted damage, and feeding preference, have noted that certain wood species are strongly feeding deterrent for termites and vary in their resistance to termite attack. Qureshi *et al*. (2012b) have reported resistance of wood species against termite attack is due to bioactive chemicals present in the wood which repel certain wood feeding insects and these toxic bioactive ingredients may kill the termites directly or indirectly by disrupting the chemistry of symbiotic microbiota (flagellates and bacteria) of termites. It is suggested that the extractives from resistant wood species may be attractive for environment and may have great market flow as future wood preservatives, if we can isolate their active ingredients by GC-MS and able to produce them economically on large scale.

The findings of feeding bioassays in laboratory and field conditions are also promising to indicate a wide range of resistance of wood species against *H. indicola*, which might be a mile stone for baiting technology and nutritional ecology of termites. Temperature can create a drying effect to the wood species which may also responsible for wood resistance and wood preference for termite. Aihetasham and Iqbal (2012) have evaluated eight commercial woods exposed to *M. championi*, and suggested that increase in temperature caused more wood degradation by termites. The present study revealed that resistance and susceptibility of wood species may prove important measures for wood prevention from termite degradation. It is also suggested from the present investigations that resistance levels among wood species may be one of the important factor for distinct division of different termite species present in same area, since division of food niches hinders interspecific competition for food and consequently, it allows overlapping of foraging territories.

The present findings revealed that among the 10 woods species experimented; the most susceptible was *P. euramericana* that can be exercised as bait attractant to enhance bait performance for control of *H. indicola* in commercial stores and household furnishers. The present study strongly recommends the analysis of extract of resistant woods to determine resistant component and its field application will be real task for eco-friendly termite control. The present study is also important to identify the naturally durable wood species which may not require any preservative treatment and may get higher preference in its commercial and household use. The present study also recommends that the natural durability is an important feature to identify and explore the wood species that can reduce the use of chemical termiticides which are expensive and harmful to environment. Furthermore, results of this study are also important to evaluate the dose levels of different preservatives for the timber protection at high termite infestation regions.

CONCLUSION

In summary, this study concluded that *D. sissoo*, *S. cumini* and *P. roxburghii* have efficient resistance against *H. indicola*. It recommends the durability that increases the use of these species as wooden structures and suppresses the exploitation of chemical pesticides against termites.

The present study also revealed that the *P. euramericana* and *B. monosperma* were most palatable woods to *H. indicola* which can be used in baiting technology to control termite infestation.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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