Research Article

Comparative Toxicity of Methanolic Extracts of some Indigenous and Exotic Flowers against Subterranean Termites *Odontotermes obesus* (Isoptera: Termitidae)

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Abstract | Subterranean termites are destructive pests and cause massive damage to agricultural crops, forest plantations, wooden infrastructures and other cellulosic products. Their control is usually done by the application of highly persistent synthetic insecticides which often cause different non-target effects such as environment contamination. This laboratory study evaluated the toxicity of methanolic extracts of eight flowers *i.e.* Mexican marigold (*Tagetes lucida*), African marigold (*Tegates erecta*), tecoma (*Tecoma stans*), calendula (*Calendula officinalis*), basil (*Ocimum basilicum*), oxeye daisy (*Leucanthemum vulgare*), lily (*Lilium longiflorum*) and chrysanthemum (*Glebionis segetum*) against the worker individuals of subterranean termite *Odontotermes obesus* (Isoptera: Termitidae). Bioassays were conducted using filter paper disc method according to completely randomized design with four replications for each treatment. Termite mortality data was recorded at 6, 12, 24 and 48 h post-treatment. Median lethal concentration (LC_{50}) and median lethal time (LT_{50}) values were calculated for each treatment using log-dose probit analysis. Results revealed a mortality response of termites directly proportional to the extract concentration for all treatments. The floral extracts of basil (*O. basilicum*) and Tecoma (*T. stans*) exhibited maximum termite mortality (*i.e.* 55.5 and 50.0%, respectively) with minimum LC_{50} and LT_{50} values, followed by the extracts of chrysanthemum (*G. segetum*) and African marigold (*T. erecta*). Overall study results suggest that above mentioned floral extracts can be further characterized for potential botanical pesticide formulations against insect pests such as subterranean termites.

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Introduction

With about 3,200 described species, termites are an important fauna of tropical and subtropical ecosystems. These edaphic invertebrates play an essential role in the organic matter decomposition and nutrients turnover in soil. However, some subterranean termite species are destructive pests of agricultural and urban settings. For instance, *Odontotermes* and *Microtermes* are two important genera of subterranean termites which cause massive damage to building materials, agricultural crops and forest plantations (Constantino, 2002; Ahmed et al., 2013).

Links Researchers

In Indo-Pak region, control of subterranean termite's infestation is primarily done by the application of different conventional synthetic insecticides (Lee and Ryu, 2003; Ahmed et al., 2006; Manzoor et al., 2012). In Pakistan, a number of synthetic insecticides are used against termites in the field or in urban settings irrespective of any scientific base, soil or structure type and termite species (Ahmed et al., 2006). Residues of most of these insecticides are highly persistent and accumulate in the soil and treated surface causing environmental contamination and human health hazards (Verma et al., 2009; Edwards, 2013).

Hence, there is a need to switch towards alternate termite control strategies which would be more biorational and environment-friendly and less-persistent than synthetic insecticides. Plant-derived extracts, for instance, emerge as promising biorational pest management tools. Botanical extracts of various plants have been found effective in controlling a wide number of insect pest species (Dodia et al., 2010). These plant extracts usually contain various metabolites such as alkaloids, flavonoids, tannins, terpenoids and saponins exhibiting anti-insect properties (Isman, 2006; Dodia et al., 2010). Some studies have shown anti-termitic properties of different plant extracts (Bläske and Hertel, 2001; Ding and Hu, 2010). However, these previous studies regarding the evaluation of insecticidal potential of botanicals have focused on the extracts of different plant parts such as roots, stems, shoots, leaves and fruits (Verma et al., 2009; Dodia et al., 2010). Very few studies have demonstrated the toxicity of flower extracts, although one study by Rao et al. (1957) showed the toxicity of petal extracts of different medicinal plants of India against rice weevil *Sitophilus oryzae*. Similarly, Badshah et al. (2004) demonstrated that crude floral extract of *Calotropis procera* was more toxic to subterranean termites (*Heterotermes indicola* and *Coptotermes heimi*) than its leave extract. Moreover, pyrethrin, a well-known natural botanical insecticide on which the synthetic pyrethroids are based, is also extracted from the petals of *Chrysanthemum cinerariifolium* flowers (Krief et al., 2009). Therefore, this laboratory study was aimed to evaluate the toxicity potential of floral extracts of eight indigenous and exotic ornamental plants against worker individuals of subterranean termite *Odontotermes obesus*.

Materials and Methods

Termite collection and maintenance

Intact parts of a subsurface colony of subterranean termite *O. obesus* were collected manually from the underside of an infested wood log and were brought to the laboratory of the Department of Entomology, College of Agriculture, University of Sargodha. For acclimatization of termite individuals to laboratory conditions, the termitarium (termite nest) parts containing hundreds of termite individuals were maintained for few days in a 1.5 ft^2 glass cube in dark conditions at 26±2°C temperature and 65±5% relative humidity. Only healthy and active termite individuals were utilized in bioassays.

Collection and extraction of flowers

Fresh flowers, cut from the pedicel level, of eight different indigenous and exotic ornamental plants, as detailed in Table 1, were collected from the flower markets of Sargodha district and were identified up to species level by an expert florist. These whole flowers were shade dried for 7 to 10 days and then were grinded into fine powder by an electric blender. Extraction of these floral powders was carried out with the help of Soxhlet apparatus in the laboratory of the Department of Food Science and Technology, University of Sargodha. For extraction, methanol was used as extraction solvent with a 1:10 (w/w) ratio and solvent was evaporated from the crude extract with the help of an electrical shaker in order to get more pure crude extracts. All floral extracts were placed in dark coloured hermetic glass vials in the refrigerator (at 4°C) until their further utilization in toxicity bioassays.

Toxicity bioassay

Methanolic floral extracts were bio assayed against *O. obesus* termite individuals using standard filter paper disc method. Treatments included four concentrations of each floral extract *i.e.* 5, 10, 20 and 40% along with one control treatment. Filter paper discs were dipped for 5 – 10 sec in the treatment solutions and were allowed to air dry for 15 to 20 min after setting them in 9 cm Perti-plates over 2-3 mm thick layer of 1.5% agar. Control treatment was composed of tab water used to prepare other treatment solutions. Ten healthy and active termite individuals (9 workers and 1 soldier) were released with camel hair brush on the treated filter paper disc in each Petri-plate and were incubated in dark at 27±2°C temperature and 65±5%

relative humidity. Data of termite mortality was recorded at 6, 12, 24 and 48 hours post-treatment. Moribund insects were counted as dead ones. Experimental design was completely randomized with five replications for each treatment.

Table 1: *Different floral species bioassayed for their toxicity potential against subterranean termites Odontotermes obesus.*

Botanical name	Common/ Vernacular name	Family	Potential Insecticidal components	Floral phenotypes used for extraction
Lilium longiflorum	Lily	Liliaceae	Kaempferol	
Tecoma stans	Tecoma	Bignoniaceae	Alkaloid, tannins, Saponins, Flavonoids	
Glebionis segetum	Chrysanthemum	Asteraceae	Luteolin, Apigenin	
Ocimum basilicum	Basil	Lamiaceace	Linalool, α – bergamotene	
Tagetes erecta	African Marigold	Asteraceae	Terpenoid, Thienyls, Ocimenone, Ocimene	
Tagetes lucida	Mexican marigold	Asteraceae	Coumarin	
Calendula officinalis	Calendula	Asteraceae	Triterpinoid, Faradiol	
Leucanthemum vulgare	Oxeye daisy	Asteraceae	Caryophyllene, Alkanoids	

Statistical analysis

Statistical analysis of data was done using Statistix® 8.1 software. Treatment means were compared using one-way factorial analysis of variance (ANOVA) followed by least significant difference (LSD) test at 0.05 level of probability. Moreover, median lethal concentration (LC_{50}) and median lethal time (LT_{50}) values were calculated by log-dose probit analysis using Polo® Software.

Results and Discussion

Results of the bioassay revealed that termite mortality was directly proportional to treatment concentration and time intervals. According to one-way factorial ANOVA, there was a significant effect of treatments ($F_{7,160}$ = 60.88; $p < 0.001$), observation time ($F_{4,160}$ = 1918.63; $p < 0.001$) and their interaction ($F_{28,160}$ = 6.55; $p < 0.001$) on the mean mortality of termite individuals exposed to different concentration of floral extracts. Maximum mean mortality (55.5%) was exhibited by the methanolic extract of *O. basilicum* (basil flowers), followed by *T. stans* (tecoma flowers) (50.50%) and *C. officinalis* (calendula flowers) (48.10%) (Figure 1). The floral extract of *T. erecta* (African marigold) was least effective against *O. obesus* individuals exhibiting 33.65% mean mortality, followed by the extracts of *L. longiflorum* (lily flowers) (39.8%) and *T. lucida* (Mexican marigold). Similar trend of termites mortality was recorded for each observation time as well (Figure 2). Maximum mortality (*i.e.* 83.5%) was recorded by the extracts of *O. basilicum* and *T. stans* at 48 h post-exposure, while minimum (*i.e.* 12.3%) was recorded for *T. erecta* extract at 6 h interval (Figure 2).

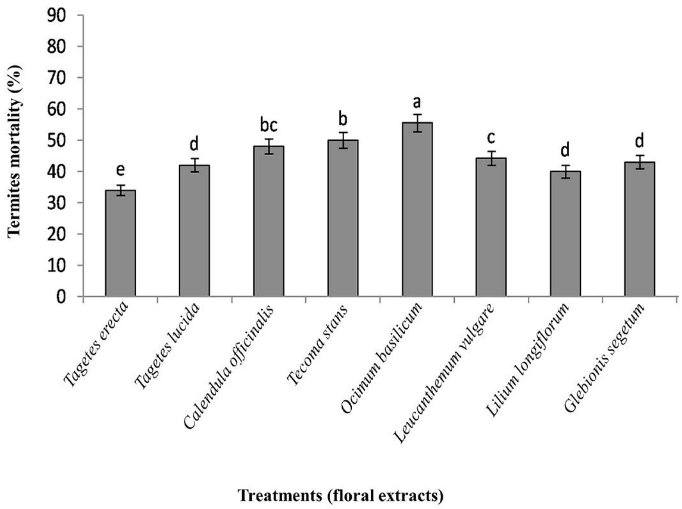

Figure 1: *Percent mortality (mean ± SE) of subterranean termite Odontotermes obesus individuals by different methanolic floral extracts. Different alphabets at column tops indicate significant differences among treatments (one-way ANOVA; LSD at α = 0.05).*

Probit analysis showed similar trend of toxicity of floral extracts as reflected by the analysis of variance. Minimum LC_{50} and LT_{50} values were recorded for all treatments respectively at maximum time interval (48 h) and maximum treatment concentration (40%). The most effective floral extracts were of *T. stans* and *O. basilicum* with minimum LC_{50} values (*i.e.* 1.8 and 4.33%, respectively) at 48 h (Table 2).

Table 2: *Median lethal concentration (LC_{50}) values of different floral extracts evaluated against subterranean termites Odontotermes obesus.*

Treatment	Time (h)	LC_{50} (%)	95% Fiducial Limit	Slope ± SE	X^2 d.f. (14)	P-value
Lilium longiflorum	12	70.8	45.4-164.7	0.95±0.10	29.9	0.079
	24	18.7	14-27.5	1.08±0.97	56.1	0.099
	48	9.9	1.0-7.4	0.96±0.97	27.9	0.060
Tecoma stans	12	80.0	50.5-170	0.95±0.10	69.7	0.85
	24	9	6.5-11.4	1.08±0.97	26.6	0.063
	48	1.8	0.2-4.4	0.96±0.97	94.8	0.031
Glebionis segetum	12	21.4	18.2-26.1	0.95±0.10	19.9	0.028
	24	5.7	7.6-98.8	1.08±0.97	20.9	0.050
	48	4.7	3.0-6.2	0.96±0.97	22.5	0.060
Ocimum basilicum	12	11.07	18.2-26.1	0.95±0.10	14.8	0.025
	24	6.1	7.6-98.8	1.08±0.97	39.3	0.052
	48	4.33	3.0-6.2	0.96±0.97	45.3	0.047
Tegates erecta	12	9.2	6.8-11.6	0.95±0.10	32.9	0.057
	24	5.8	4.2-7.2	1.08±0.97	24.9	0.038
	48	4.93	3.4-6.2	0.96±0.97	40.5	0.043
Tagetes lucida	12	22.3	15.5-20.4	0.95±0.10	44.7	0.54
	24	10.5	8.9-12.1	1.08±0.97	13.8	0.023
	48	6.8	5.4-8.1	0.96±0.97	24.8	0.028
Calendula officinalis	12	26.4	22.1-33	0.95±0.10	14.8	0.028
	24	14.1	11.5-17.3	1.08±0.97	22.9	0.044
	48	5.1	2.6-7.4	0.96±0.97	38.4	0.097
Leucanthemum vulgare	12	21.8	18.8-25.9	1.17±0.09	15.0	0.023
	24	10.3	1.21-31.1	1.11±0.08	15.5	0.026
	48	5.8	4.2-7.3	1.4±0.10	34.3	0.042

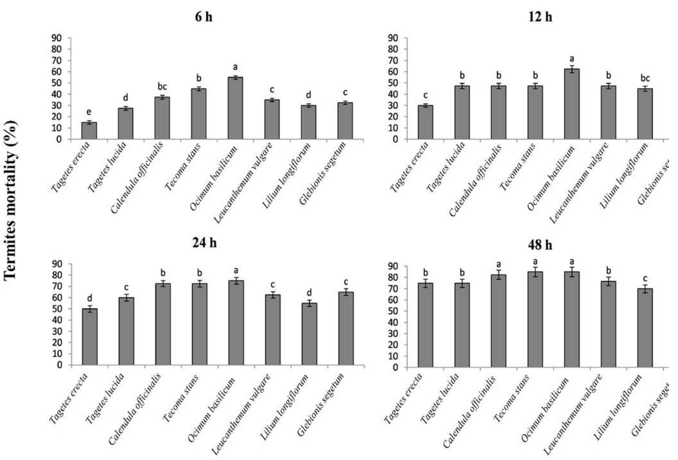

Figure 2: *Percent mortality (mean ± SE) of subterranean termite Odontotermes obesus individuals by different methanolic floral extracts at different time intervals. For each time interval, different alphabets at column tops indicate significant differences among treatments (one-way ANOVA; LSD at α = 0.05).*

However, minimum LT_{50} values of 5.7 and 4.5 h were recorded for *T. erecta* (African marigold) extract at 20 and 40% concentration, respectively, followed by *O. basilicum* (*i.e.* 6.7 and 4.6 h, respectively) (Table 3).

Table 3: *Median lethal time (LT_{50}) values of different floral extracts evaluated against subterranean termites Odontotermes obesus.*

Treatment	Conc. (%)	LT_{50}	95%FL	Slope±SE	X^2 (d.f 10)	P-value
Lilium longiflorum	20	28.1	5.6-21.8	1.2±0.16	28.7	0.13
	40	16.1	9.8-21.8	1.4±0.15	40.1	0.15
Tecoma stans	20	12.6	6.9-16.9	1.11±0.15	33.2	0.20
	40	11.4	7.9-14.2	1.99±0.17	28.6	0.06
Glebionis segetum	20	11.1	6.2-14.8	1.12±0.15	26.8	0.16
	40	4.7	1.12-9.8	0.9±0.16	13.8	0.14
Ocimum basilicum	20	6.7	2.8-9.9	1.17±0.16	25.9	0.16
	40	4.6	3.8-7.8	2.7±0.25	26.9	0.07
Tegates erecta	20	5.7	1.9-9.0	1.03±0.16	22.6	0.18
	40	4.5	0.85-8.0	1.5±0.19	33.9	0.17
Tagetes lucida	20	13.3	9.8-16.3	1.2±16.3	11.5	0.20
	40	9.3	6.4-11.8	1.5±0.17	14.2	0.06
Calendula officinalis	20	16.5	2.7-11.1	1.07±0.15	18.4	0.11
	40	8.3	3.3-12.1	1.07±0.15	19.3	0.13
Leucanthemum vulgare	20	18.5	10.2-25.9	1.18±0.15	42.3	0.23
	40	8.6	2.8-12.8	1.2±0.16	31.6	00.17

Subterranean termites have been destructive pests of many agricultural and horticultural crops and of wooden infrastructures all over the world. Termite control primarily relies on the utilization of synthetic insecticides that can retain in the environment for a longer period of time such as different organochlorines and organophosphate formulations (Rao et al., 2005; Ahmed et al., 2006). Therefore, there is a need of seeking biorational termite control strategies such as botanical extracts which can be safe and environment-friendly. Different plant based compounds may exhibit insecticidal, repelling and/or antifeedant activities against different insect pests including termites and can be used as alternate to the synthetic insecticides (Zhu et al., 2001; Isman, 2006; Ahmed and Qasim, 2011).

This study evaluated the methanolic floral extracts of eight different plants against the worker and soldier individuals of subterranean termite *O. obesus*. Results showed that extracts of *O. basilicum* and *T. stans* are comparatively more effective than other treatments. Sweet basil (*O. basilicum* is an aromatic medicinal herb and has been extensive studied for its anti-insect activities against different insect pests such as stored grain insect pests (Popovic et al., 2006), mosquitos (Umerie et al., 1998; Sundararajan et al., 2018) and lepidopterous caterpillars (Kostić et al., 2008; Pandir et al., 2016). On the other hand, only limited number of data is available regarding the insecticidal activity of *T. stans* extracts against different insect pests. Roa et al. (1957) documented the toxicity potential of kerosene extract of *Tecoma indica* flowers against rice weevil *S. oryzae*. The extract of *T. stans* leaves have been demonstrated effective against larvae of *Aedes aegyptii* and *Culex quinquefasciatus* (Navaneethan et al., 2016; Hari and Mathew, 2018) and against stored grain weevils (Abere and Enoghama, 2015). Similarly, Sakthivadivel and Daniel (2008) demonstrated the mosquitocidal activity of the petroleum ether extract of *T. stans* flowers.

Conclusions and Recommendations

Subterranean termites (*O. obesus*) are destructive urban and agricultural pests being primarily controlled by persistent synthetic insecticides. The present study was aimed to evaluate the toxicity potential of methanolic extracts of eight different flowers against these termites. Results revealed that among all treatments the extracts of *O. basilicum* (basil) and *T. stans* (tecoma) flowers exhibited maximum mortality for treatment after 72 hours of application was exhibited by basil extract (55.5%) followed by tecoma (50.00%), calendula (48.00%) and others with decreased mortality. LC_{50} values were inversely proportional to time interval while LT_{50} values were found minimum at 40% concentrations for all treatments. Basil and tecoma found to be most effective floral extracts among all the utilized treatments.

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Authors Contribution

Muhammad Shahzad Akbar performed experiments and prepared manuscript, Maria Aslam performed experiments and data analysis, Muhammad Rehan Khalid prepared samples and helped in writing first draft of the MS, Shahid Iqbal provided technical assistance in experimentation, Muhammad Luqman performed statisical analyses and did proof-reading of the manuscript and Muhammad Zeeshan Majeed conceived and designed the experimental protocols and made technical revision of manuscript.

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