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EVALUATION OF SELECTED BOTANICAL EXTRACTS AGAINST MENDI TERMITE *Macrotermes subhyalinus* (Isoptera: Termitidae), UNDER LABORATORY CONDITION

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ABSTRACT

Termites are most pestiferous insects causing damage to crop and buildings. Their control still relies mainly on harmful chemical pesticides to the detriment of eco-friendly pesticides. The main objective of the study was evaluate seed extracts of *Brassica nigra* and leaves extracts of *Acokantra schimperi*, *Croton macrostachyus* and *Rhamnus prinoides* against *Macrotermes subhyalinus*, known to cause damage to crops, vegetation and buildings in Ethiopia. Treatments were consisted of three concentrations levels (5, 10 and 15 weight of botanical powder (g) per 100 ml volume of water) by three replications. Mortality of termite was counted after 24, 48 and 72 hours exposure for both conditions. The results of all botanical extracts at all concentration levels showed that caused mortality of *M. subhyalinus* workers. Complete mortality (100%) of *M. subhyalinus* was observed after treatment with 15 w/v *B. nigra* extract at three time intervals. Moreover, *A. schimperi* at 15 w/v concentration also resulted 100% mortality after 48-72 hours of exposure. *Brassica nigra* extract showed least LC_{50} (5.63g/100ml) value than other botanical extracts after 24 hours exposure. Based on their toxicity status extracts of *B. nigra* > *A. schmperi* > *R. prinoides* > *C. macrostachyus* leaf extracts.

Contribution/Originality: This study contributes to the existing literature by giving important information for farmers, government and Non-governmental developmental sectors to control Mendi Termite Macrotermes subhyalinus by using of these locally available botanical extracts alternatives rather than using synthetic pesticides because, botanical extracts have minimum mammalian toxicity and environmental influences.

1. INTRODUCTION

Termites are eusocial insects that are the most agriculturally and structurally important insects and which cause for vast economic loss by feeding on many crops, plants and wooden strictures in buildings. They have ability to feed various stages of plant growth [1]. Termites are successful and destructive pests that comprise under order Isoptera, that developed cellulose and xylan digestion capabilities that allow them to obtain energy and nutrition from nutritionally poor food sources, such as plant material and residues derived from it (e.g., wood and humus) [2] which, damage homes and other structures in short period of time, because their colony members are huge and long lived [3]. To control termites, synthetic pesticides play important roles. However, Intensive use of synthetic insecticides is one of the strongest factors responsible for the rapid development of resistance in many species of insects and create number of ecological problems, development insect resistance and unsafe to non-target organisms

including human being [4]. Using synthetic pesticides repeatedly to manage termites increase environmental influences, pest resistance and pest resurgence of other insect pests [5].

These adverse effects of synthetic pesticides are bearing attention for development of botanical pesticides to control different insect pests. Botanical extracts and powders from different bioactive plants used for insecticidal, repellent and anti-feeding properties [6]. Plant based pesticides are preferred to control insect pests because of their less harmful nature to non-target organisms due to their innate biodegradability [7]. The uses of these biologically active botanical extracts are usually safer to humans and the environment than conventional pesticides, with minimal residual effects and also with least development of resistance against pests. Therefore, the aim of this study was to evaluate the efficacy of *B. nigra* seeds and *A. schimperi*, *C. macrostachyus* and *R. prinoides* leaves extracts against *M. subhyalinus* workers under laboratory condition.

2. MATERIALS AND METHODS

The study design: Laboratory experiment was arranged as Complete Randomized Design (CRD) with three replications per treatment for each botanical extracts concentration levels. The same batch of *M. subhyalinus* workers for laboratory experiment was chosen and collected from newly termite infested field (February-May, 2017) by bucket traps. All botanical extracts effectiveness was evaluated against workers by comparing from standard check (Chloropyrifos 48% E. C) and an untreated check (water).

Collection of plant materials: For efficacy test four locally available botanicals Table 1 were selected based on cultural used information and preliminary study. They collected from Debre Markos town and surrounding from January 21st to 25th in 2017.

Plant materials were brought into the laboratory by polyethylene bags. Identification of botanical species has been done by expert. Voucher specimens of the botanical species were deposited in herbarium at Debre Markos University, Ethiopia.

Table-1. List of plant materials tested against *M. subhyalinus* termite workers.

Scientific name	Family name	Common name	Part used
Brassica nigra	Brassicaceae	Black mustard	Seeds
Acokanthera schimperi	Apocynaceae	Poison bush	Leaves
Croton macrostachyus	Euphorbiaceae	Broad levead-croton	Leaves
Rhamnus prinoides	Rhamnaceae	Dogwood	Leaves

Source: Medicinal plant in Ethiopia.

Preparation of botanical extracts: Collected botanical parts were washed by sterilized water to avoid dust particles and tiny organisms that are present on them. After that, they were dried in well-ventilated room under shade for ten days until completely dry at room temperature (18-25°C). Dried form of plant materials were grinded into powder by using pestle and mortar to obtain uniform fine powder and stored in separate plastic containers. Sensitive balance was used to measure 5g, 10g and 15g portion of each grinded fine powder. Each grinded botanical powder were soaked in 100 ml of water to obtain three concentration levels (5, 10 and 15 w/v) weight of powder/volume of water, then shacked for five minutes to make homogeneous. After stay two days, each solution supernatant was filtered by Whatman (No.1) to remove impurities. Filtration has been done repeatedly to obtain maximum amount of the extract. Then, 25ml extract solution was taken and applied independently for each treatment. Chloropyrifos 48% Emuisifiable Concentrate was diluted in water based on the recommended field application rate. In all experiments, the same volume (25ml) of Chlorpyrifos 48% E.C. and water were used as a standard check and untreated check controls, respectively. The percentage of extracts varies, when the amount of material used were transformed, in same water quantity as described [8]. Efficacy of selected botanical extracts against *M. subhyalinus* under laboratory condition: Laboratory experiment was done repeatedly to check the accuracy. Bioassays were carried out to determine the mortality of Formosan workers by each botanical termiticides

activities. For laboratory test, 42 cups were labeled and arranged in carton box (120cm x 80cm x 40cm), which was inspected for maintenance of required moisture level. The volume of each container was 1 liter. Then, a piece of moistened Eucalyptus globules wood was added as food source and five gram soil to maintain moisture level were placed for survival of termites in each prepared cups. Immediately, 20 workers were counted and introduced in each 42 prepared cups carefully. For acclimatization of termites, laboratory room temperature (20-28°c) was controlled by using 100 watt electric bulb. Moisture also adjusted by added drops of water in each cups to create optimum humidity. The box had covered by sake that able to exchange of air for them and stay for five days to check their adaptation in the laboratory. During application time, from each prepared filtered solution 25ml extract was sprayed in each cup to test the efficacy. Mortality of termites was recorded carefully and accurately after 24, 48 and 72 hours of exposure. Percentage of mortality has been calculated by Abbott [9] equation.

$$\text{Percent mortality} = \frac{\text{No. of dead termite}}{\text{Total No. of termite}} \times 100$$

Data analysis: Data was subjected to one-way of analysis of variance (ANOVA) by using SPSS version 20 software to determine statistically significant differences among treatments. Significant differences means were separated by using Tukey's studentized range test (α =0.05). The lethal concentration (LC_{50}) and (LC_{90}) were analyzed by probit analysis to determine botanical extracts toxicity. 50% and 90% mortality were calculated after 24, 48 and 72 hours.

3. RESULTS AND DISCUSSION

Evaluation of the efficacy of botanical extracts under laboratory conditions: Effectiveness of botanical extracts on of *M. subhyalinus* workers under laboratory condition represented in Table 2. As indicated in Table 2, at 5 w/v botanical extracts concentration level after 24 hours exposure highest and lowest mortality were recorded from *B. ngra* and *R. prinoides* respectively under laboratory condition. Similarly, after 48 hours exposure of time interval maximum termite mortality (100%) also was recorded from standard check.

At 5 w/v botanical extracts concentration level, after 48 hours exposure mean mortality of termites was increased as 66.67, 33.33, 10.53 and 23.33 mean mortality of termites were recorded from *B. ngra*, *A. schmperi*, *C. macrostachyus* and *R. prinoides* respectively. At 5 w/v concentration level of botanical extracts, the highest (78.33) and the least (26.67) mean mortality of termites under laboratory condition were registered from *B. nigra* and *C. macrostachyus* respectively, after 72 hours exposure time.

Table-2. Mean mortality ± SE of of *M. subhyalinus* workers by botanical extracts under laboratory.

Treatments	Concentration (w/v)	Mean mortality ± SE over time (hours) exposure		
		24 hours	48 hours	72 hours
Brassica nigra	5	53.33 ± 1.67^{d}	66.67 ± 1.67^{d}	78.33 ± 1.67^{c}
	10	86.67 ± 1.67^{b}	90.00 ± 0.00^{b}	96.67 ± 1.67^{a}
	15	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Acokanthera schmperi	5	21.67 ± 1.67^{g}	33.33 ± 2.89^{f}	41.67 ± 1.67^{e}
	10	53.33 ± 3.33^{d}	63.33 ± 1.67^{d}	76.67 ± 1.67^{c}
	15	91.67± 2.89^{b}	100.00±0.00^{a}	100.00 ± 0.00^{a}
Croton macrostachyus	5	8.33 ± 1.67^{h}	18.33 ± 2.89^{g}	26.67 ± 1.67^{f}
	10	30.00 ± 2.89^{f}	36.67± 1.67^{f}	40.00 ± 0.00^{e}
	15	43.67 ± 1.67^{e}	53.33 ± 3.33^{e}	61.67 ± 2.89^{d}
Rhamnut prinoides	5	11.67 ± 1.67^{h}	23.33 ± 1.67^{g}	31.67 ± 1.67^{f}
	10	56.67 ± 1.67^{d}	65.00 ± 0.00^{d}	73.33 ± 1.67^{c}
	15	78.33 ± 1.67^{c}	83.33 ± 1.67^{c}	88.33 ± 1.67^{b}
Chloropyrifos 48% E.C	25ml	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Untreated check (water)	25ml	0.00 ± 0.00^{i}	0.00± 0.00^{h}	0.00± 0.00^{g}
CV (%)		5.30	3.67	3.54
P-value		<0.0001	<0.0001	<0.0001

Means within a column followed by the same letter are not significantly different (Tukey Student Test (HSD) at p=0.05). Key: SE=Systematic Error, w/v= weight of powder/volume of water.

Mean mortality of termite (96.67%) by *B. nigra*, (76.67) mortality by *A. schmperi* (40.00)% by *C. macrostachyus* and 73.33 by *R. prinoides* were recorded after 72 hours at 10 w/v concentration. There was highly significant difference (P<0.0001), among the different treatments at different time intervals (24, 48 and 72 hours) due to the potency (termicidal effect) of botanical extracts. There was no significant difference among *B. nigra* at 5 w/v, *A. schmperi* and *R. prinoides* at 10 w/v concentration levels after all (24, 48 and 72 hours of exposure time as indicated in Table 2.

Maximum termite mortality (100%) from Chloropyrifos followed by non-significantly difference 10 and 15 w/v concentration of *B. nigra* and 15 w/v concentration of *A. schmperi* after 72 hours exposure under laboratory condition. As indicated in Table 2, 61.67 and 88.33 mean mortality of termites were recorded from *C. macrostachyus* and *R. prinoides* respectively at 15 w/v concentration level after 72 hours exposure of time.

The highest and least mean mortality of termite was recorded from *B. nigra* seeds and *C. macrostachyus* leaf extracts respectively. No termite mortality was recorded from untreated check. Mean mortality of termites in all botanical extracts concentration levels was also increased based on exposure time intervals.

Table-3. Toxicity analysis of botanical extracts on of *M. subhyalinus* workers.

Treatments	After 24 hours		After 48 hours		After 72 hours	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
B. nigra	5.63	9.71	4.86	8.75	3.97	6.87
A. schmperi	9.20 (7.60-10.91)	14.72 (12.66-18.57)	7.65 (6.15-9.17)	12.45 (10.66-15.77)	6.65 (5.15-8.09)	11.14 (9.45-14.24)
C. macrostachyus	15.31 (12.51-22.50)	24.64 (19.16-43.01)	13.44 (10.90-18.64)	23.15 (18.16-37.66)	11.91 (9.56-15.84)	21.44 (17.04-33.04)
R. prinoides	10.36 (8.66-12.78)	16.42 (14.08-21.05)	8.65 (6.95-10.36)	14.52 (12.42-18.34)	7.98 (6.24-9.69)	13.98 (11.88-17.77)

Source: SPSS statically procedures.

Dose response bioassay under laboratory condition: The results of the LC_{50} and LC_{90}, is indicated in Table 3. Minimum concentration required to kill 50% and 90% of the tested worker of *M. subhyalinus* were calculated for each botanicals after 24, 48 and 72 hours exposure of time with their lower and upper limits. As indicated in Table 3 minimum concentration (3.97g/100ml) that used to kill 50% of tested termites' recorded from *B. nigra* after 72 hours. Maximum concentration (15.31 g/100ml) that used to kill 50% of tested termites' recorded from *C. macrostachyus* after 24 hours exposure.

In the present study, tested botanical extracts showed mortality on the of *M. subhyalinus* workers. As indicated in Table 2 there was highly significant difference (P<0.0001) between different treatments at different time of exposure (24, 48 and 72 hours) due to the potency of botanical extracts at all concentration levels. There was no mortality of termites observed in the untreated check over the entire exposure (72 hours) of the experiment. Maximum mean mortality of termites (100%) was registered from Chloropyrifos 48% E. C, which highly significantly differences from botanicals treatments that cause mortality against *C. formosanus* workers. This is in agreement with Shiberu, et al. [10] reported that Chloropyrifos 48% E. C gives 100% mortality on *Macroterms* Spp. under laboratory condition.

The present experiment showed that, mean mortality of termites was increased, when the concentration level of botanicals were increased and time of exposure increased Table 2. Based on the present results, maximum termite mortality was observed at higher concentrations (15 w/v) after 72 hours exposure. This result is in agreement with Upadhyay [11] and Sattar, et al. [12] who reported that mortality of termites, were directly proportion to botanical extract concentrations and exposure time of treatments.

In the present study, mean mortality (100%) on of *M. subhyalinus* workers by positive control was not significantly different from 15 w/v *B. nigra* after all time of exposure and *A. schmperi* after 48 and 72 hours exposure

time Table 2. In the current study, *B. nigra* aqueous seeds extract (LC_{50}= 5.63 g/100ml water and LC_{90}=9.71 g/100ml) displayed highest lethal effect against of *M. subhyalinus* workers after 24 hours exposure.

In others investigation, 70% ethanol *B. nigra* seeds extract, possessed good microbial activities [13]. Whereas water extracts of elder flowers of *Sambucas nigra* can repel granary weevils (*Sitophilus granarius*) at 5, 10 and 15 w/v concentrations in 200 ml water [14]. In the present study, *C. formosanus* termite mortality was recorded in seed extract of *B. nigra* and leaf extracts of *A. schmperi*, *C. macrostachyus* and *R. prinoides* after 24 hours exposure under laboratory condition (LC_{50}=5.63, 9.20, 15.31 and 10.36g/100ml) and (LC_{90}= 9.71, 14.72, 24.64 and 16.42g/100 ml water) respectively.

In present experiment, *C. macrostachyus* leaf extracts at 5, 10 and 15 w/v concentration levels after all time of exposure showed less toxic effect among all botanical extracts. This result was in lined with the finding of Shiberu, et al. [10] who reported that seed extracts of *C. macrostachys* at 25% concentration level was also less toxic effect on *Macroterms Spp.* (Isoptera: Termitidae), even after 5 days under laboratory conditions.

Croton macrostachyus leaf extract of the present study gave 26.67% and 15.00% mortality on *C. formosanus* workers after 72 hours exposure at 5 w/v concentrations under laboratory and semi-field conditions respectively. While in the other findings, aqueous leaf extracts of *C. macrostachyus* at 5 w/v concentration level achieved 100% mortality on African bollworm, *Helicover pagrmiyera* Hubner after 72 hours [15].

4. CONCLUSION

The present study findings had important implications in the practical control of Mendi Termite by using botanical extracts, which are easy to prepare. From the present study results, it can be concluded that, the efficacy of botanicals depending on the amount of concentration and time of application (acting in short, medium and long period of time after treated). The result showed that, *B. nigra* and *A. schmperi* were more effective than other treatments on *M. subhyalinus* workers. So, users use high concentration of effective botanicals for monitoring or preventing Mendi termite workers in short period of time. From the above result, it is clear that all the tested botanical extracts at 15 w/v concentration level after 72 hours provided more than 50% mortality. As a result, *B. nigra*, *A. schmperi*, *C. macrostachyus* and *R. prinoides* botanical extracts can be used as an integrated termite management (IPM) at higher concentration levels. In conclusion, this study considering the bio-activity of extracts from seeds of *B. nigra*, leaves of *A. schmperi*, *C. macrostachyus* and *R. prinoides* at managing of *M. subhyalinus* termite workers could be considered for exploration in the management of Mendi termites on the field.

5. RECOMMENDATIONS

Aqueous extract of tested botanicals have promising. Therefore, wide range of field study should be made particularly in areas where Mendi Termite species is highly prevalent to determine the practical potential of the botanical extracts by using different solvents like ethanol, methanol, distilled water, etc. at different concentrations. Further studies should be made on the evaluation of these plants extract on other termite species and insect pests on the field. This study also suggest that active ingredient of botanical extracts responsible for causing mortality of Mendi Termite *Macrotermes subhyalinus* workers should be identified and non-target effects of botanical extracts should be studied.

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