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**RESEARCH ARTICLE**

# Green synthesized silver nanoparticles for controlling subterranean termites, *Psammotermes hypostoma* (Desneux)

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## Abstract

Subterranean termites *Psammotermes hypostoma* (Desneux) has become a major pest in Egypt. To investigate new effective and inexpensive ways of control this pest, we assessed nanoparticles synthesized by a green approach using corresponding salt (sliver nitrate) with aqueous extract of garlic, chili, turmeric, and black pepper were commercially purchased. UV–visible wavelength scanning and nanoparticle tracking analyses were used to determine the quantity and quality of nanoparticles. The experiments were carried out to evaluate the toxicity of aqueous plant extracts and their green synthesized silver nanoparticles compared with mineral oil, *Beauveria bassiana*, and chlorpyrifos on *P. hypostoma*. The result showed that chlorpyrifos was the most toxic compound, whereas black peper aqueous extract was the least toxic one, however, *B. bassiana*, K. Z. oil, Garlic aqueous extract, Chili pepper AgNPs, turmeric aqueous extract, Turmeric AgNPs, Garlic AgNPs, Black peper AgNPs, and Chili pepper aqueous extract lie in between. Conclusively, based on those results all selected compounds and extracts caused significant mortality for workers of *P. hypostoma* as compared to control and can be used as a cheap and effective alternative in future IPM programs against *P. hypostoma.*

**Keywords:** Termites; insecticide; alternatives; extract; nanoparticles.

## 1. Introduction

In Egypt eight species of subterranean termites were detected. Among all species, *Psammotermes hypostoma* Desneux (Isoptera: Rhinotermitidae) is the most destructive subterranean termites that inhabit the soil in urban, rural and agricultural areas in Egypt essentially in upper Egypt and the new valley (Ahmed *et al.*, 2014). In the last years, the subterranean termites caused great damage to many houses and buildings in Qena and Aswan governrate, (Ahmed and Mohany, 2008).

Termite management has always relied entirely on chemicals, particularly synthetic pesticides (Nisar *et al.*, 2012). The development of insecticide resistance in target pests, as well as detrimental effects on human health and worries about environmental deterioration, are some of the drawbacks that prevent pesticides from being widely used (Rice and Coats, 1994). The solution to this problem lies in finding and developing a rational environmental strategy for the management of pests and which is referred to as IPM. Therefore, persistent need is to find alternative ways to manage termites to be safe, cheap and available. The demand for environmentally friendly nanoparticle production processes has resulted in a rise in interest in biological approaches that avoid the use of harmful chemicals and the formation of toxic byproducts. There is a growing demand for 'green nanotechnology' in this regard (Daohua *et al.*, 2011). One of the new technologies used for

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pest control, which has become one of the new techniques for pest control in recent years, nanotechnology technique (Bhattacharyya *et al.*, 2010). Nanotechnology technique used to control termites and protect the wood from termite attacks. Also using Nano-ZnO resulted in protective wood from termite, which was repelled effect to termite, (Clausen *et al.*, 2011). Green synthesis outperforms chemical and physical processes in terms of cost, environmental friendliness, ease of scaling up for large-scale synthesis, and the absence of high pressure, energy, temperature, or harmful substances. Environmentally friendly, yet chemically complex, bio reduction of metal ions by combinations of biomolecules employing microbes, biological control, enzymes, and plant extracts. (Song and Kim, 2008; Narayanan and Sakthivel, 2010; Rastogi and Arunachalam, 2011; Abhishek *et al.*, 2021) Plant-mediated synthesis appears to be a preferred approach among the many green synthesis methods since it produces stable nanoparticles in less time and facilitates steady synthesis. (Iravani, 2011). Qena governrate is one of most infested localities with subterranean termites. The present investigate was focused to find out the insecticidal potential of fresh garlic (*Allium sativum* L.) fruits, chili pepper (*Capsicum sp.* L.), Turmeric (*Curcuma longa*) and black pepper (*Piper nigrum*) extracts and their biosynthesized $Ag^+$ nanoparticles compared to synthetic insecticides and mineral oils on subterranean termite *P. hypostoma*.

## 2. Materials and Methods

### *2.1. Study area and chemicals*

This study was carried out at Plant Protection Dept., Faculty of Agriculture, South Valley University, Qena, Egypt.

In this study, the alternative control of subterranean termite *P. hypostoma* was carried out by using aqueous plant extracts and their green synthesized silver nanoparticles compared with K.Z. oil (mineral oil), Bio-fly (biopesticide), and Chlorpyrifos insecticide (chemical pesticide) as recommended by the Egyptian ministry of agriculture (ARAPC, 2021) table (1).

**Table 1.** Insecticide, aqueous extracts and silver nanoparticles extracts for the selected compound against subterranean termites (*P. hypostoma*)

| Trade name | Abbv.* | Active ingredient | Rate |
|---|---|---|---|
| Chlorpyrifos | Cho | Chlorpyrifos | 100-80-60-40-20% of the crude compounds |
| KZ Oil 95% EC | KZ | Mineral oil | |
| Bio-fly | Bio | *Beauveria bassiana* (1 $cm^3$ contain 30 $\times 10^6$ cell) | |
| Garlic fruits aqueous extract | GaEx | *Allium sativum* (Crude extract) | |
| Garlic AgNPs | GaNPs | Nano particles | |
| Chili pepper fruits aqueous extract | ChiEx | *Capsicum sp.* (Crude extract) | |
| Chili pepper AgNPs | ChiNPs | Nano particles | |
| Turmeric fruits aqueous extract | TuEx | *Curcuma longa* (Crude extract) | |
| Turmeric AgNPs | TuNPs | Nano particles | |
| Black pepper fruits aqueous extract | BpEX | *Piper nigrum* (Crude extract) | |
| Black pepper AgNPs | BpNPs | Nano particles | |

* abbreviations

### *2.2. The insect collection*

The subterranean termites, *P. hypostoma* (Desn). were collected from the agricultural reasearth farm of Faculty of Agriculture, South Valley University, from July to August 2020 using (EL-Sebay, 1991). An area about $150m^2$ heavlly infested by subterranean termite *P. hypostoma* was chosen for this study. the holes were digged with small axe, where the holes arranged in 6 rows and 5 columns, the distance between holes was 2 cm and the holes deepth was 12 cm. the traps prepared in the laboratory and then were send to the experimental area, all traps where waitted with water and distributed randomly all over the area. Traps were removed from the infected sites and transported to the laboratory after fifteen days for bioassay. The workers were transferred from traps to glass aquarium and provided with pieces of wet cardboard as a source of cellulose and humidity for five days in an incubator adjusted at 27 ±1°C for their survival assurance. Daily inspection and eliminated for dead or moribund individuals, healthy workers were used for the evaluation.

### 2.3. Pesticides and plant extracts

Bioassay assessment carried out using eleven pesticides and their alternatives: Chlorpyrifos, (Bio-fly), K.Z. oil, aqueous Garlic extract, Garlic silver nanoparticles, aqueous Chili extract, Chili silver nanoparticles, aqueous Turmeric extract, Turmeric silver nanoparticles, aqueous Black pepper extract, and Black pepper silver nanoparticles.

### 2.4. Preparation of Plant Extract

Fresh garlic (*Allium sativum*) fruits, Chili pepper (*Capsicum* sp.), Turmeric (*Curcuma longa*) and Black pepper (*Piper nigrum*) were bought commercially, washed and air dry for a week at room temperature, then ground into fine powder using tissue grinder (IKA A10, Germany) and stored in a dry airtight container. 100 gm of powder was dissolved in 1000 ml sterile distilled water, shacked using shaker 180 rpm for 24 hrs. The obtained extract was filtered using Whatman No.1 filter paper, the filtrate collected in 1000 Erlen-meyer flask and then stored at 4º C until used later modified from (Verástegui *et al.,* 1996).

### 2.5. Biosynthesis of nano-scale silver particle

With continuous stirring 100 ml of $AgNO_3$ (2 mM) solution freshly prepared were added drop-wise to 100 ml of the stored aqueous extracts at 50-60º C for reductions of $Ag^+$ ions. The resulted solutions were incubated in a dark room at 37º C until being used (Mondal *et al.,* 2011).

### 2.6. Characterization of nano-scale silver nanoparticles

#### 2.6.1. UV–visible spectra analysis

The UV–visible spectrum analysis of silver nanoparticles (AgNPs) of aqueous garlic, chilli, turmeric, and black peper was performed using the optical density (OD) "Shimadzu UV-2401 PC, Japan" scan spectrophotometer. Between 200 and 800 nm, measurements were taken at a resolution of 1 nm and a scanning speed of 300 nm/min. The UV–vis spectra of 1 ml aliquots of the sample and 2 ml deionized as water were measured in a quartz cell to assess the reduction of Ag+ ions (Wiley *et al.,* 2006). Silver nitrate (2mM) was employed as a blank to change the baseline.

#### 2.6.2. Transmission electron microscopy (TEM)

After the reaction, the precipitate sank to the bottom of the conical flasks, and the suspension above was sampled for transmission electron microscopy (TEM) examination. The size and form of extract nanoparticles were investigated at 70 kV using a "LEOL-2010, Japan" transmission electron microscope (TEM) equipped® with a digital "Kodak Megaplus 1.6i camera" and picture analysis and processing software (AMT, USA). As previously described, the sample was pared by placing a drop of each solution onto a carbon-coated copper grid and drying it at room temperature (Sathishkumar *et al.,* 2009). The size distribution of the obtained nanoparticles was measured using TEM micrographs.

#### 2.6.3. Fourier Transform Infra-Red Spectroscopy (FTIR) spectra

The FTIR spectra of AgNPs were recorded at room temperature using a Perkin–Elmer spectrophotometer in the range 4000–400 $cm^{-1}$. The UV140404B spectrophotometer was used to record diffuse reflectance spectra in the 200–800 nm wavelength range, and numerical data was plotted in the 'Origin 7' programme (Slman *et al.,* 2018).

#### 2.6.4. X-Ray Diffraction (XRD) analysis

After (XRD) analysis, the resultant mixture of silver nanoparticles was centrifuged at 10,000 rpm for 30 minutes. The residues of

AgNPs were washed twice with double distilled water at 80ºC to prepare powder AgNPs for X-ray powder diffraction investigations. Powder X-ray diffraction (XRD) patterns were collected using copper radiation (Cu Ka, 1.5406) at 40 kV and 30 mA on a (Shimadzu XRD-6000) (Slman *et al.*, 2018).

### *2.7. Bioassay*

The evaluation of tested compounds was conducted by five concentrations 100, 80, 60, 40 and 20 ml/L for each treatment, distilled water used as a control. Ten workers were placed in 120 mm Petri dishes using aspirator to avoid direct contact and to avoid crushing insects, adding treated pieces of cardboard (2*2 cm) with previous concentrations, then kept in an incubator at 27±1°C. Mortality of workers was recorded after 24 hours, using a needle to poke the insect to be sure if it is still alive.

### *2.8. Data analysis*

For toxicity index, all the mortality data were statistically analyzed according to (Finney, 1971). Toxicity lines were drawn on probit-log paper and the median lethal concentration $LC_{50}$ and slope values were calculated by computerized probit analysis program LdP Line Program, (Bakr, 2005).

Toxicity index (T.I.): was calculated for each insecticide according to the equation of (Sun, 1950) as follow:

T.I. = ($LC_{50}$ of the most toxic insecticide / $LC_{50}$ of other tested insecticide) x 100.

Relative toxicity (R.T.) Folds: These values were measured according to the equation of (Metcalf, 1967) as follow:

R.T. = ($LC_{50}$ of the lowest toxic insecticide / $LC_{50}$ of other tested insecticide) x 100.

## 3. Results and Discussion

### *3.1. Tests proving the formation of AgNPs*

The detailed study on the biosynthesis of silver nanoparticles by fresh Garlic (*Allium sativum*) fruits, Chili pepper (*Capsicum sp*.), Turmeric (*Curcuma longa*) and Black pepper (*Piper nigrum*) was employed and reported in this work. The aqueous silver ions were reduced to silver nanoparticles when added to tested plant extracts. It was observed that the color of the solution for GaNPs, ChNPs, TuNPs, and BpNps turned from (white to pale brown), (red to dark brown), (yellow to dark brown) and (black to pale grey), respectively after 24 hours prior to the reaction, which indicated the formation of silver nanoparticles. UV–vis spectrophotometer analysis was used to track the formation and stability of reduced silver nanoparticles in colloidal solution. The greatest absorbance at 420 nm was seen in the UV–vis spectra, which increased with the time being spent incubating silver nitrate with the plant extract (Fig 1, 2, 3 & 4).

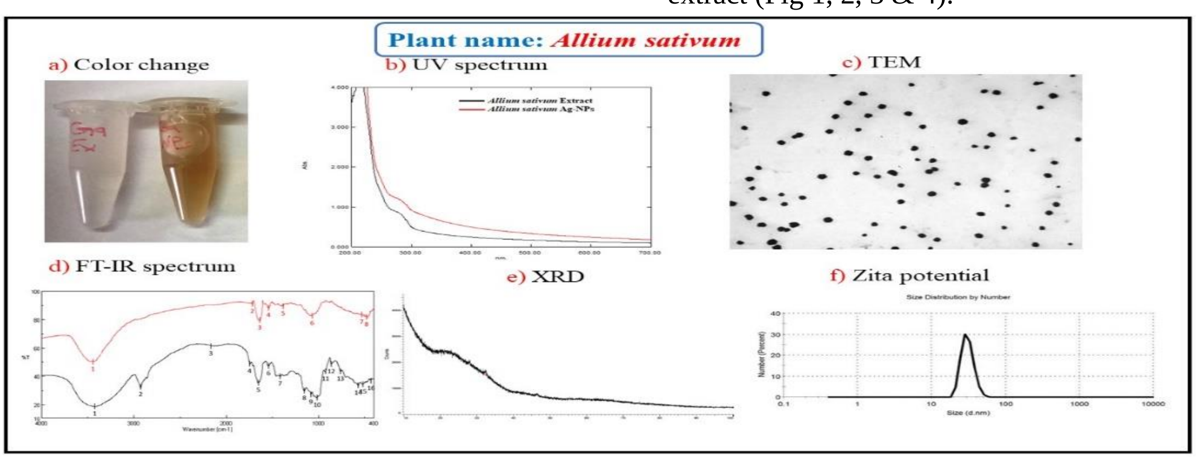


**Figure 1.** Tests of silver nanoparticles formation by aqueous extract of fresh Garlic (*Allium sativum*) fruits.

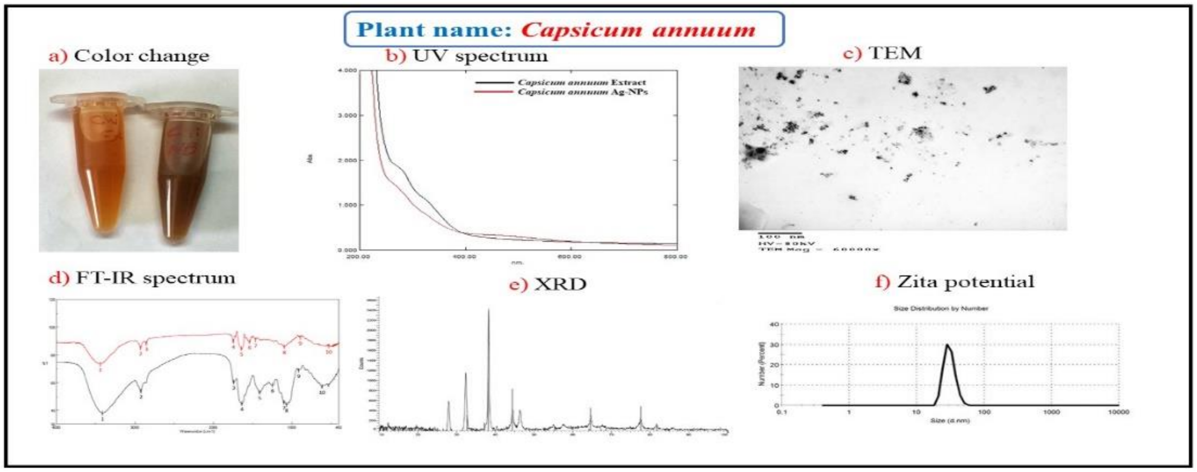


**Figure 2.** Tests of silver nanoparticles formation by aqueous extract of Chili pepper (*Capsicum sp*.)

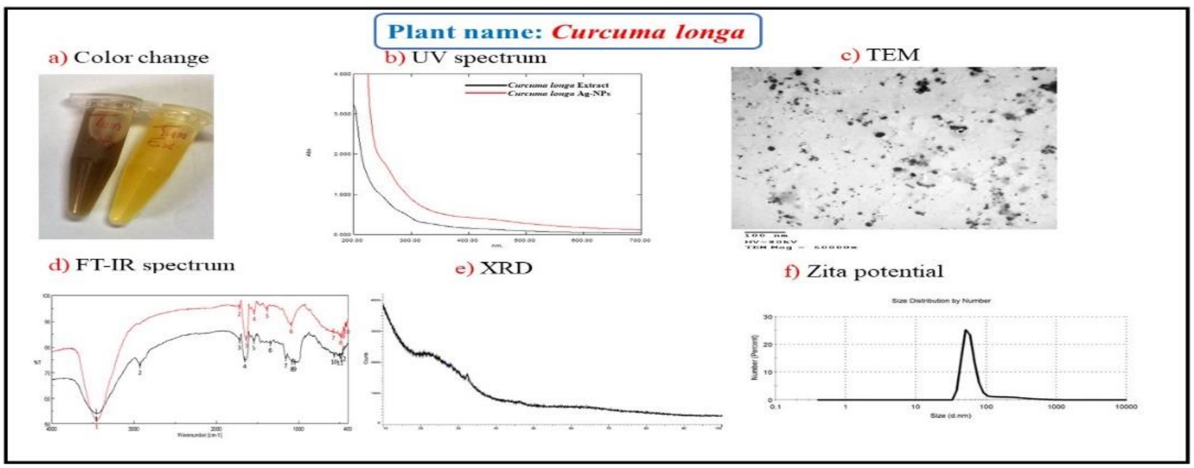


**Figure 3.** Tests of silver nanoparticles formation by aqueous extract of Turmeric (*Curcuma longa*)

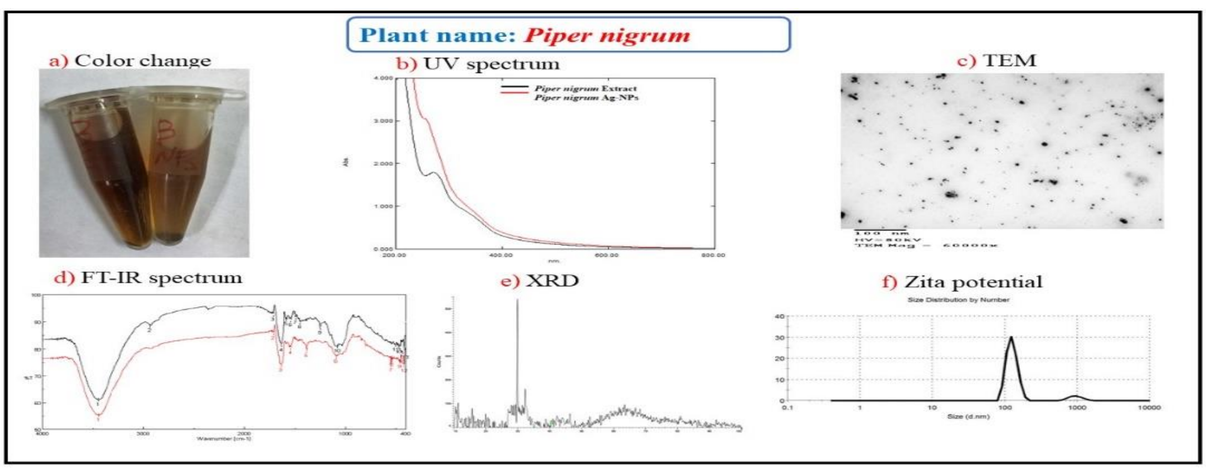


**Figure 4.** Tests of silver nanoparticles formation by aqueous extract of Black pepper (*Piper nigrum*)

The existence of some functional groups and the dual activity of the plant extract as a reducing and capping agent were confirmed by FTIR analysis of silver nanoparticles. The size, shape, and morphology of nanoparticles have been determined using transmission electron microscopy (TEM). It demonstrates that the silver nanoparticles are well disseminated and mostly spherical in shape, with some NPs having irregular shape structures, as seen in (Fig 1, 2, 3, &4).

### *3.2. Toxicity study*

Cho, Bio, K.Z, GaEx, GaNPs, ChiEx, ChiNPs, TuEx, TuNPs, BpEx, and BpNPs, were tested and evaluated to determine their impact on *P. hypostoma* at the laboratory condition.

Data in Table (2) and Fig. (5) represented the relative toxicity of tested compounds against *P. hypostoma*. Data indicate that the order of efficiency of the tested compounds was the same at both $LC_{50}$ and $LC_{90}$ levels. The tested insecticides could be descending arranged as follows: Cho, Bio, K.Z, GaEx, ChiNPs, TuEx, TuNPs, GaNPs, BpNPs, ChiEx, and BpEx. The corresponding $LC_{50}$ values were 4.71, 29.69, 34.98, 44.39, 49.41, 50.71, 51.14, 52.72, 64.97, 89.81 and 121.62 ppm, while the $LC_{90}$ values were 57.21, 153.15, 86.76, 124.14, 117.64, 129.82, 106.08, 122.53, 143.64, 195.36 and 403.33 ppm, respectively. On the other hand, $\chi 2$ values were 0.47, 1.55, 4.31, 0.42, 1.77, 6.08, 2.28, 6.44, 3.46, 7.04, and 6.29, respectively.

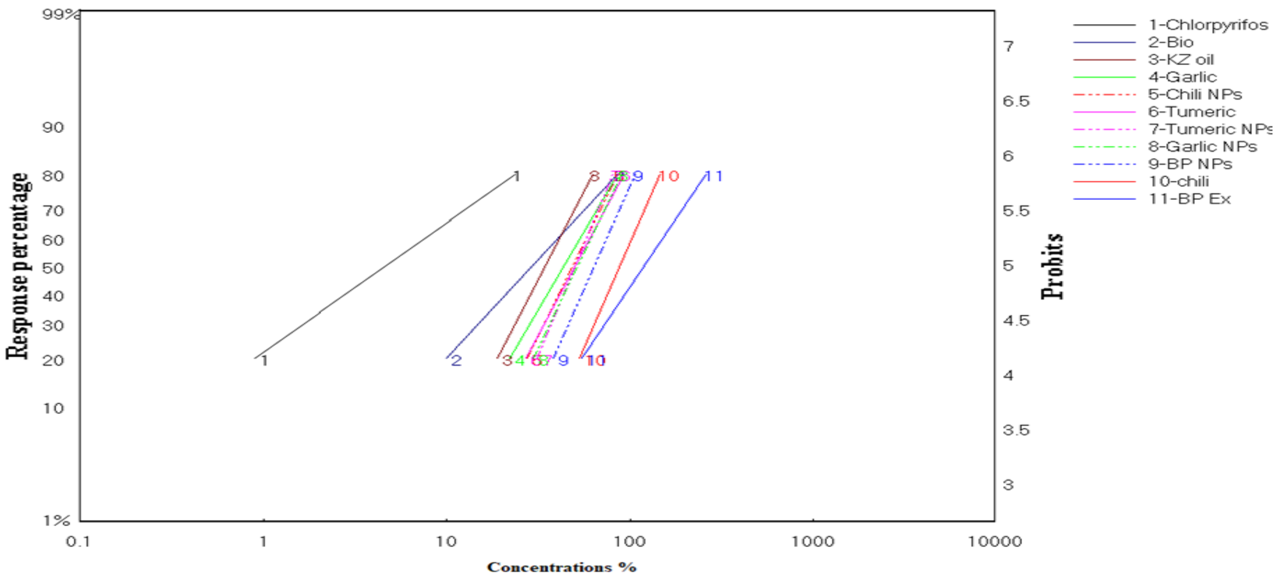


**Figure 5.** Toxicity of eleven pesticides and their alternatives against *P. hypostoma*.

When comparing the confidence limits and their overlapping with others, it was obvious that the confidence limits are overlapped with Bio, K.Z, GaEx, ChiNPs, TuEx, TuNPs, GaNPs, BpNPs, ChiEx, and BpEx, but not overlapped with Cho. Thus, we can say there is no significant difference between Bio, K. Z, GaEx, ChiNPs, TuEx, TuNPs, GaNPs, BpNPs, ChiEx, and BpEx, but they are significantly different with Cho Fig (5), it is also obvious, as shown in Table (2) and Fig. (5), that Cho had the steepest toxicity line and BpEx had the flattest, however Bio, K. Z., GaEx, ChiNPs, TuEx, TuNPs, GaNPs, BpNPs and ChiEx lie in between. This reflects the superiority of Cho insecticide and inferiority of BpEx. Cho was the most toxic compound, whereas BpEx was the least toxic one.

Data in table (2) show that Bio, K. Z, GaEx, ChiNPs, TuEx, TuNPs, GaNPs, BpNPs and ChiEx and BpEx were 6.30, 7.43, 9.43, 10.49, 10.77, 10.86, 11.19, 13.79, 19.07 and 25.83 as toxic as Cho at the $LC_{50}$ level, respectively on ground of potency levels (Relative activity). Cho, Bio, K.Z, GaEx, ChiNPs, TuEx, TuNPs, GaNPs, BpNPs, and ChiEx were 100, 15.86, 13.46, 10.61, 9.53, 9.29, 9.21, 8.93, 7.25, and 5.24 folds as effective as BP Ex, respectively, at the $LC_{50}$ level, respectively. These results agreed with those of Chen *et al.* (2015) study effect ten insecticides belong different group on the subterranean termite *Reticulitermes speratus*. The $LC_{50}$ values of the insecticides were determined by exposing workers to insecticide-treated sand. Results showed the insecticides in order of decreasing toxicity to the termite species Bbifenthrin > Phoxim > Chlorpyrifos > Cypermethrin > Carbofuran > Fipronil > Propoxur > Ivermectin > Iimidacloprid > Abamectin. Also, the toxicity insecticide groups ranking of groups in order of decreasing toxicity to the termite: organophosphate > Carbamate > Pyrethroid > Neonicotinoid > Phenyl pyrazole > Avermectin. On the other hand, (Park and Shin, 2005) Garlic oil (3.5 micro liter/liter) was tested against the Japanese termite *Reticulitermes spertus* Kolb, with 100% mortality rates after 24 hours of treatment, which matches our findings perfectly. Among the plant essential oils, garlic oil has the most potent anti-termite acidity. Also (Shi *et al.*, 2004; Tuan *et al.*, 2014) reported that in comparison to untreated control, sprayed garlic and chilli combination + soap 0.1 percent was very effective in controlling insect pest cabbage vegetable. As reported by Ahmed *et al.* (2016), Garlic caused 100% mortality after only 24 hours against *Heteroterms indcila*. Furthermore, Abhishek *et al.* (2021) reported that scientists have explored farmers friendly strategies like cultural, physical, mechanical, biological control (entomopathogenic fungi, bacteria, nematodes, botanical extracts etc.) having broad applicability and potential to control termites. By utilizing entomopathogenic agents as a component of IPM approach one can get better results. Also, Richa *et al.* (2021) detected the insecticidal activities of clove leaf essential oils, Garlic essential oil, Neem Oil, Orange Essential oils against the adult termites it was examined under different concentrations. It was concluded that by increasing the concentration the mortality rate was increased. The result showed that clove oil and garlic oil was proved to be very effective at little concentrations also.

**Table 2.** Toxicity of tested compounds against *P. hypostoma.*

| Compounds | $\chi^2$ | $LC_{50}$ *ppm | Confidence limits of $LC_{50}$ Lower | Confidence limits of $LC_{50}$ Upper | $LC_{90}$ *ppm | Slope | Index (T.I.) | Folds (R.T.) |
|---|---|---|---|---|---|---|---|---|
| Cho | 0.47 | 4.71 | 0.33 | 10.75 | 57.21 | 1.18 ±0.34 | 100 | 1.00 |
| Bio | 1.55 | 29.69 | 16.25 | 39.82 | 153.15 | 1.79 ±0.44 | 15.86 | 6.30 |
| K. Z | 4.31 | 34.98 | 27.69 | 41.52 | 86.76 | 3.25 ±0.51 | 13.46 | 7.43 |
| GaEx | 0.42 | 44.39 | 35.71 | 52.98 | 124.14 | 2.87 ±0.49 | 10.61 | 9.43 |
| ChiNPs | 1.77 | 49.41 | 41.49 | 57.62 | 117.64 | 3.40 ±0.53 | 9.53 | 10.49 |
| TuEx | 6.08 | 50.71 | 42.22 | 59.82 | 129.82 | 3.14 ±0.52 | 9.29 | 10.77 |
| TuNPs | 2.28 | 51.14 | 43.94 | 58.48 | 106.08 | 4.04 ±0.59 | 9.21 | 10.86 |
| GaNPs | 6.44 | 52.72 | 44.71 | 61.33 | 122.53 | 3.49 ±0.55 | 8.93 | 11.19 |
| BpNPs | 3.46 | 64.97 | 56.18 | 76.04 | 143.64 | 3.72 ±0.62 | 7.25 | 13.79 |
| ChiEx | 7.04 | 89.81 | 77.04 | 115.86 | 195.36 | 3.79 ±0.78 | 5.24 | 19.07 |
| BpEx | 6.29 | 121.62 | 91.63 | 248.65 | 403.33 | 2.46 ±0.64 | 3.87 | 25.83 |

T.I. - Index compared with chlorpyrifos R.T. - No. of folds compared with BP Ex $\chi^2$ = Chi-square * = ppm based on a:i

## 4. Conclusion

Based on the findings, it is feasible to suggest that, in addition to mineral oil, aqueous plant extracts, and their green synthesised silver nanoparticles, *Beauveria bassiana* as a biopesticide should be included in IPM programmes for the control of the subterranean termite *P. hypostoma*.

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*All authors are contributed in this research.*

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*There is no fund in this research.*

**Institutional Review Board Statement**

*All Institutional Review Board Statement are confirmed and approved.*

**Data Availability Statement**
*Data presented in this study are available on fair request from the respective author.*
**Ethics Approval and Consent to Participate**
*This work carried out at plant protection department and followed all the department instructions.*
**Consent for Publication**
*Not applicable.*
**Conflicts of Interest**
*The authors declare no conflict of interest.*

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