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RESEARCH ARTICLE

Toxicity of certain traditional and bio-insecticides against subterranean termite, *Psammotermes hypostoma* Desneux using controlled cardboard-dip bioassay.

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Abstract

Subterranean termite, Psammotermes hypostoma Desneux causes valuable economic damage in cellulose materials in Aswan governorate, Egypt. The present work was conducted to evaluate the toxicity of seven chemical and certain plant extracts namely, chlorpyrifos, emamectin benzoate, deltamethrin, fipronil, imidacloprid, spinetoram diflubenzuron and entomopathogenic fungi (Metarhizium anisopliae, Beauveria bassiana) and conventional plant extract (chili extract, garlic extract) and nano silver plant extract (chili extract AgNps, garlic extract AgNps) against subterranean termite, Psammotermes hypostoma using cardboard-dip bioassay technique under laboratory conditions. The results show that, the ranke of tested insecticides against P. hypostoma after 24, 48 and 72 h has the same descending order as follow chlorpyrifos> emamectin benzoate> deltamethrin> fipronil> imidacloprid> spinetoram> diflubenzuron> chili extract AgNPs> garlic extract AgNPs> chili extract > garlic extract. On the other hand, the fungi M. ansiopliae was more effectiveness than B. bassiana against P. hypostoma which the mortality rates after 7day were 25% and 17.5% to M. anisopliae and B. bassiana, respectively. After 14 days of treatments with increased period the result shows the mortality rates were 38.3%and 29.1% to Metarhizium anisopliae and Beauveria bassiana, respectively. While, after 21 days of treatment, the results show the mortality rates were 55.8% and 48.3% to M. ansiopliae and B. bassiana, respectively. Several new insecticides are promising in control termites with chlorpyrifos like emamectin benzoate, a biopesticides. Further, studies should be done about the mixture effect of chlorpyrifos with plant extracts and bioagents for reducing the hazard effects of chlorpyrifos insecticide.

Keywords: Subterranean termite; Insecticide; Plant extract; Entomopathogenic fungi; Cardboard-dip bioassay

1. Introduction

Subterranean termite, *Psammotermes hypostoma* Desneux (Isoptera: Rhinotermitidae) has become one of the most economic urban pests in Egypt due to the large damage of the buildings, rural brick mud, timber farmed and furniture (Ahmed and El –Sebay, 2008). In the recent years, the subterranean termites caused high damage for many houses and buildings in several Egyptian regions like Cairo, Alexandria, Port-Said, Damanhur, New Valley, Assiut, Qena, Luxor, Aswan, and Sinai (Ahmed and Mohany, 2008; El-Bassiouny and El-Rahman, 2011; Ahmed *et al*., 2014; Ahmed *et al*., 2015; Ghesini and Marini, 2017). The use of chemical insecticides is of great important for termite's control. Chlorpyrifos consider one of these insecticides which are highly toxic and recommended to control subterranean termites in Egypt (World Health Organization, 2020).

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The extensive usage of these insecticides led to the emergence of the pest resistance. The use of the new insecticide groups (i.e., neonicotinoids) to avoid the development of the pest resistance is recommended (Ahmed and Matsumura, 2012). The toxicity of this new chemical insecticide group to mammals and birds is generally low (Ahmed and Qasim, 2011). It is essential to find alternative ways to manage termites these ways must be safe, cheap and available (Logan *et al.*, 1990). Biological control by using fungi which is very effective on many types of termites (Milner, 2003). The mode of action of an entomopathogen is mine because it is possible for a small amount of vaccine to spread all over the nest before discovery, resulting in an epidemical condition (Kramm *et al*., 1982). Lenz (2005) mention that *B. bassiana* and *M. anisopliae* can operate with the impact of slow acting as biocontrol agent on the termite community. Nano silver plant extracts give a vast, virtually untapped reservoir of chemical compounds with a lot of potential uses. One of these uses is in agriculture to control pests with less risk than with synthetic insecticides that are toxicologically and environmentally undesirable. A lot of experiments using plant extracts in human and animal health protection, agriculture and household pest control have been particularly promising (Scott *et al.*, 2004). The effect of insecticides, plant extracts and entomopathogenic fungi were poorly studied on subterranean termites, *P. hypostoma* in Aswan governorate, Egypt. Therefore, the present investigation may help in achieving a successful control programme for checking of ravages of this pest. The aim of these studies is to compare the toxicity and effectiveness of tested insecticides and conventional plant extracts, chili extract (*Capsicum sp*.) and garlic extract (*Allium sativum)* and nano silver plant extracts namely, garlic extract AgNPs and chili extract AgNPs and entomopathogenic fungi, *M. anisopliae* and *B. bassiana* against subterranean termites using cardboard-dip bioassay technique under laboratory conditions.

2. Materials and methods

2.1. Termite Collections

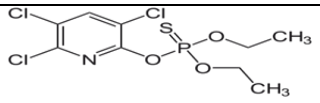
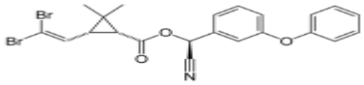
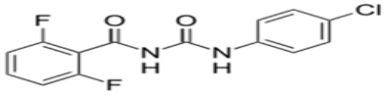
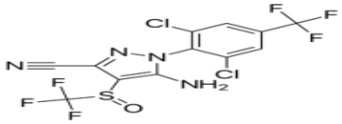
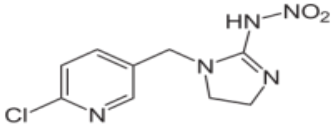
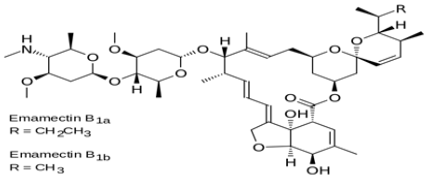
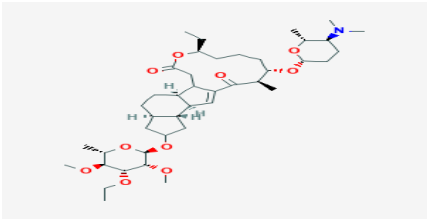
Field strains of subterranean termite, *P. hypostoma* were collected by El-Sebay trap, (El-Sebay, 1991) (Fig 1.) from the infested locations (Sahari, Aswan University and Kima region) in Aswan governorate. The four traps sent to the site of infection after fifteen days, traps were removed from the infected sites then, transferred to the laboratory of Plant Protection Department, Faculty of Agriculture, Aswan University. The individuals were removed from the trap or cardboard rolls by using a small soft brush and kept in Petri-dish (9 cm diameter) provided with moistened corrugated cardboards as a source of cellulose and moisture, with the necessary termites' humidity for three days in incubator adjusted at 25±2°C. The daily inspection carried out and eliminated dead or moribund individual. The healthy termite workers (nearly at the same size and shape) were used in the bioassay.

Fig 1. El-Sebay traps.

2.2. Chemical insecticides

Seven commercial insecticides were used, chlorpyrifos **(**New Turbofos 50% EC), imidacloprid **(**Imida plus 35%SC), fipronil (Fipromex 20 %SC), deltamethrin (Grap 2.5 %EC), diflubenzuron (Newbenzeron 48%SC), spinetoram (Radiant 12% SC), emamectin benzoate (Opal 5.7%EC). The common name, chemical structure shown in **Table 1.**

Table 1. Insecticides tested in the toxicological experiments against subterranean termite, *P. hypostoma.*

No.	Common name	Group	Chemical structure
1.	Chlorpyrifos	Organophosphates (OP)	 O.O-diethyl O-3.5.6 - trichloro-2-pyridyl phosphorothioate
2.	Deltamethrin	Pyrethroids	 [(S)-Cyano-(3-noxyphenyl)- methyl](1R,3R)-3-(2,2-bromoethenyl)-2,2-dimethyl-cyclopropane -1-carboxylate
3.	Diflubenzuron	Insect growth regulators	 N-[(4-Chlorophenyl) carbamoyl] -2,6- difluorobenzamide
4.	Fipronil	Phenylpyrazoles	 (RS)-5-Amino-1-[2,6-dichloro -4-(trifluoromethyl) phenyl] -4-trifluoromethylsulfinyl) pyrazole-3-carbonitrile
5.	Imidacloprid	Neo-nicotinoids	 N-{1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl }nitramide
6.	Emamectin benzoate	Avermectins	 4″-Deoxy-4″-epi-methylaminoavermectin B1;Epimethylamino-4″-deoxyavermectin
7.	Spinetoram	Spinosyns	 2R,5R,9R,10S,14R,15S,19S)-15-[(2R,5S,6R)-5-(dimethylamino)-methyloxan-2-yl]oxy-7-[(2R,3R,4R,5S,6S)-4-ethoxy-3,5-dimethoxy -6-methyloxan-2-yl]oxy-19-ethyl-14-methyl-20-oxatetracyclo[10.10.0.02,10.05,9]docos-11-ene-13,21-dione

2.3. Plant-extracts

2.3.1. Preparation of conventional extracts

Fresh fruits garlic (*Allium sativum*), chili pepper (*Capsicum sp.),* 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 one hundred gm of powder was dissolved in 1000 ml distilled water, shacked using shaker 180 rpm for 24 h The obtained extract was filtered using Whatman No.1 filter paper, the filtrate collected in 1000 Erlen-meyer flask and then stored as stock solution (100.000 mg L^{-1}) at 4ºC until used later modified from (Verástegui *et al*., 1996).

2.3.2. Biosynthesis of Nano-scale silver particle

According with, nano silver of the tested extracts prepared in pesticides labs at Faculty of Agriculture, South Valley University, Qena governorate. 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 50-60ºC for reductions of Ag^+ ions. The resulted solutions were incubated in a dark room at 37ºC until being used (Mondal *et al.,* 2011).

2.4. Characterization of nano-scale silver nanoparticles

2.4.1. UV–visible spectra analysis

UV–visible spectral analysis, for silver nanoparticles (AgNPs) of aqueous garlic and chili was carried out using the optical density (OD) "Shimadzu UV-2401 PC, Japan" scanning spectrophotometer. Measurements were performed between 200 and 800 nm with a resolution of 1 nm and a scanning speed of 300 nm/min. The reduction of Ag^+ ions was monitored by measuring of the UV–vis spectrum of 1 ml aliquots of the sample and 2 ml deionized as water in quartz cell indicated earlier (Wiley *et al.,* 2006). Used Silver nitrate (2mM) was to adjust the baseline as a blank.

2.4.2. Transmission electron microscopy (TEM)

The precipitate had settled at the bottom of the conical flasks and the suspension above was sampled for transmission electron microscopy (TEM) observation after the reaction. Processing software (AMT, USA), the size and shape of extracts nanoparticles were observed at 70 kV using "LEOL-2010, Japan" transmission electron microscope (TEM) equipped® with digital "Kodak Mega plus 1.6i camera" and image analysis. The sample was pared by placing a drop of each solution on a carbon-coated copper grid and drying in room temperature as previously described by (Sathishkumar *et al*., 2009). The size distribution of the resulting nanoparticles was estimated based on TEM micrographs.

2.4.3. Fourier Transform Infra-Red Spectroscopy (FTIR) spectra

Diffuse reflectance spectra were recorded using UV140404B spectrophotometer in the wavelength range 200–800 nm and numerical data were plotted in the 'Origin 7' software, (FTIR) spectra of AgNPs were recorded at room temperature on Perkin–Elmer spectrophotometer in the range 4000–400 cm^{-1} (Slman *et al*., 2018).

2.4.4. X-Ray Diffraction (XRD) analysis

(XRD) analysis, the solution of the developed nanoparticles of silver was centrifuged at 10,000 rpm for 30 min. The solid residues of AgNPs were washed twice with double distilled water and then dried at 80 ºC to obtain powder AgNPs used for X-ray powder diffraction measurements. The powder X-ray diffraction (XRD) patterns were recorded on (Shimadzu XRD-6000) with copper radiation (Cu Ka, 1.5406 Å) at 40 kV and 30 mA (Slman *et al*., 2018).

2.5. Entomopathogenic fungi

M. anisopliae and *B. bassiana* were used in the bioassay were brought from Egyptian Association for Sustainable Agriculture in Cairo, Egypt.

2.6. Cardboard-dipping laboratory bioassay

Efficacy of insecticides against subterranean termite, *P. hypostoma* five

concentrations of aqueous solution of each pesticides (0.1, 1, 10, 100, 1000 mg L^{-1}) were used for each insecticides used. Cardboards (5 x 5 cm) were dipped in tested concentration for 10 seconds and left to dry under laboratory conditions for about half an hour, and then placed in Petri-dish (9 cm diameter). Ten healthy termite workers (same size and shape) with three replicates of the same termite colony were put in incubator adjusted at 25±2°C. Control cardboards were similarly dipped in a solution of distilled water only. For the plant extracts, five concentrations (100.000, 80.000, 60.000, 40.000, 20.000 mg L^{-1}) were used in a bioassay. The tested termite mortality was recorded after 24, 48 and 72 h of treatment. A termite worker considered dead if it was incapable of coordinated forward movement.

For the tested entomopathogenic fungi, three concentrations (32×10^8, 16×10^8 and 8×10^8 conidia/ml) were used in afore mentioned above. The tested termite mortality was recorded after 7, 14 and 21 day of treatment.

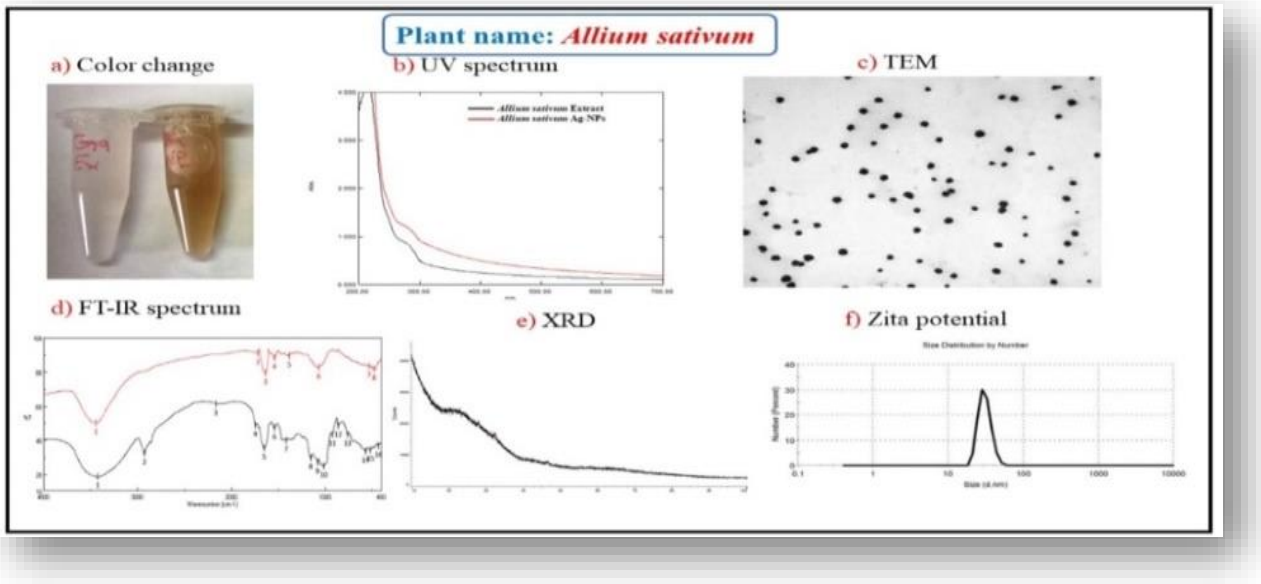


Fig 2. Tests of silver nanoparticles formation by aqueous extract of fresh garlic *(Allium sativum)* fruits.

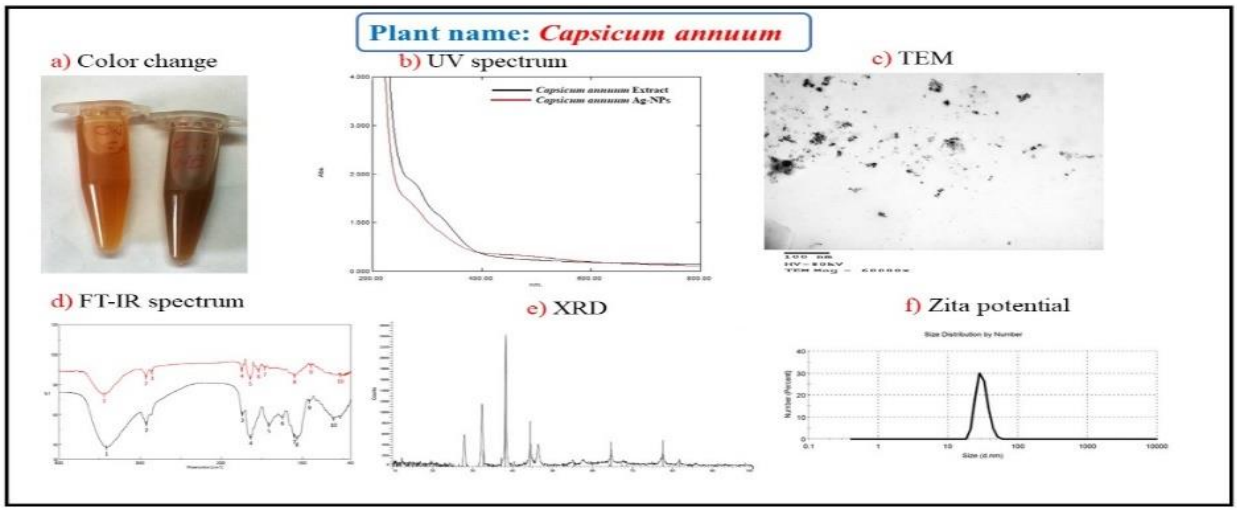


Fig 3. Tests of silver nanoparticles formation by aqueous extract of chili *(Capsicum sp.).*

2.7. Koch's hypothesis tests for the M. anisopliae and B. bassiana

The dead cadavers were transferred into new Petri-dish with witted filter paper. These plates incubated to observe the outgrowth of fungus. The fungal growth was isolated to insure the specific species. Where a white mold appeared that produces many dry, powdery conidia in white spore balls and this is called a fungus *B. bassiana*. While a green color appeared from the fungus spores due to the green muscarinic disease that it cause to the insect as a results of penetration of the cuticle and it is called a fungus *M. anisopliae*

2.8 Data analysis

Mortalities were calculated for each concentration, and they were corrected for normal mortality (control) with Abbott's correction as the following: Abbott corrected mortality (%Mortality in treatment-% Mortality in control/ 100 - %Mortality in control) ×100 (Abbott, 1925).

The values of the LC_{50} and toxicity index were calculated using SPSS (2017) (Version 16.0 for windows, SPSS Inc., Chicago, the USA) and Ldp line program for insecticides. Ldp line program were used to estimate the values of the LC_{50} and toxicity index for nano silver plant extracts and fungi. Toxicity lines were drawn on probit-log paper and the median lethal concentration LC_{50} and slope values were determined.

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.

3. Results and discussion

3.1. Toxicity of tested insecticides and plant extracts against P. hypostoma

Results in Table 2 and Fig. 4 represent the toxicity of emamectin benzoate, deltamethrin, fipronil, imidacloprid, spinetoram, diflubenzuron, conventional extract (chili extract and garlic extract) and nano silver plant extract (chili extract AgNps and garlic extract AgNps) compared with chlorpyrifos against *P. hypostoma* after 24, 48 and 72 h. using cardboard-dip bioassay. Based on the LC_{50} values after 24, 48 and 72 h. for the tested materials, the most toxic insecticide was chlorpyrifos (38.19, 8.67 and 0.30 ppm) followed by emamectin benzoate (77.06, 22.25 and 1.39 ppm), deltamethrin (128.56, 37.69 and 2.02 ppm), fipronil (299.41 , 42.48 and 3.67 ppm), imidacloprid (396.74 ppm, 67.39 ppm and 4.87 ppm), spinetoram (880.94 , 92.42 and 9.3 ppm), diflubenzuron (1204.21 , 144.06 and 21.34 ppm), chili extract AgNps (51865.3, 49097.3, 17102.9 ppm), garlic extract AgNps (110320, 59312.9, 22208.9 ppm), chili extract (156120, 84243.7, 35249.2 ppm) and garlic extract (178219, 105230, 49065.4 ppm), respectively**.** These results show that chlorpyrifos was the most potent against subterranean termite, *P. hypostoma* among all tested insecticides and plant extracts and show that chlorpyrifos insecticides had the highest toxicity and diflubenzuron had the least insecticide tested. Based on the confidence limits and their overlapping on the tested materials no significant difference among the tested insecticides at 24, 48 and 72h. Many studies agreement with present study, Ahmed *et al.* (2014) studied the toxicity of chlorpyrifos 48% EC and imidacloprid 20% SL on the subterranean termite, *P. hypostoma*, after 3, 6, 12 and 24 h using the cardboard-dip bioassay. The LC_{50} value of chlorpyrifos was 28.29 ppm and 0.36 ppm at 3 and 24 h and imidacloprid showed the most toxic neonicotinoid pesticide with LC_{50} value 50.95 ppm after 3 h and decreased to 0.82 ppm after 24 h of treatment. chlorpyrifos exhibited the most potent pesticide among the pesticides used, whereas imidacloprid was the high potent among the neonicotinoid pesticides against *P. hypostoma*, while Abd-Ella (2020) showed that

chlorpyrifos has the most insecticides activity, with LC_{50s} 1.19 and 0.13 ppm, followed by imidacloprid with LC_{50s} 3.24 and 0.61 ppm, while spinosad was the least toxic one with LC_{50s} 9.96 and 4.95 ppm.

The tested plant extracts had the lowest effects compared the tested insecticides. There is a significant difference in LC_{50s} values of tested insecticides and the tested extracts, however no significant difference among the tested extracts. Chili extract AgNps was more effective than garlic extract AgNps, chili extract and garlic extract exhibited the lowest values of LC_{50} in all treatments. Chili extract AgNps had the highest toxicity and garlic extract had the least one. This reflects the superiority of chili extract AgNps and in feriority of garlic extract AgNps. These results are in the same line with the results reported by Iqram *et al*. (2003) who tested 10 different plant extracts and antagonistic fungi for the manager of *xanthomonas compastris*. The authors found that garlic extract gave the highest results against this pest. Park and Shine (2005) tested garlic oil (3.5 micro liter) on the Japanese termite, *Reticulitermes spertus* Kolb. Garlic oil shows the best effective anti-termite acidity among the plant essential oils, with 100% mortality rates after 24 h of treatment. Green and Arango (2007) evaluated five commercial products were tested in order to explore a broad range of formulation and silver forms: colloidal, ionic and nano particles against eastern subterranean termites, *R. flavipes*. Results showed several formulations to have excellent capacity to limit termite feeding and wood mass loss during the 4-week test: silver dispersion, zinc nanoparticles plus silver and silver protein plus N'N-hydroxynaphthalamide (NHA). It is not clear if silver is the primary active component in all these formulations. Mode of action of these plant extracts on termites has effects oviposition and AChE inhibitory activities. Garlic essential oils can be used as contact toxicity, fumigant toxicity, repellent, oviposition inhibitory and developmental inhibitory (Chaubey, 2017).

3.2. Efficiency of two entomopathogenic fungi against P. hypostoma

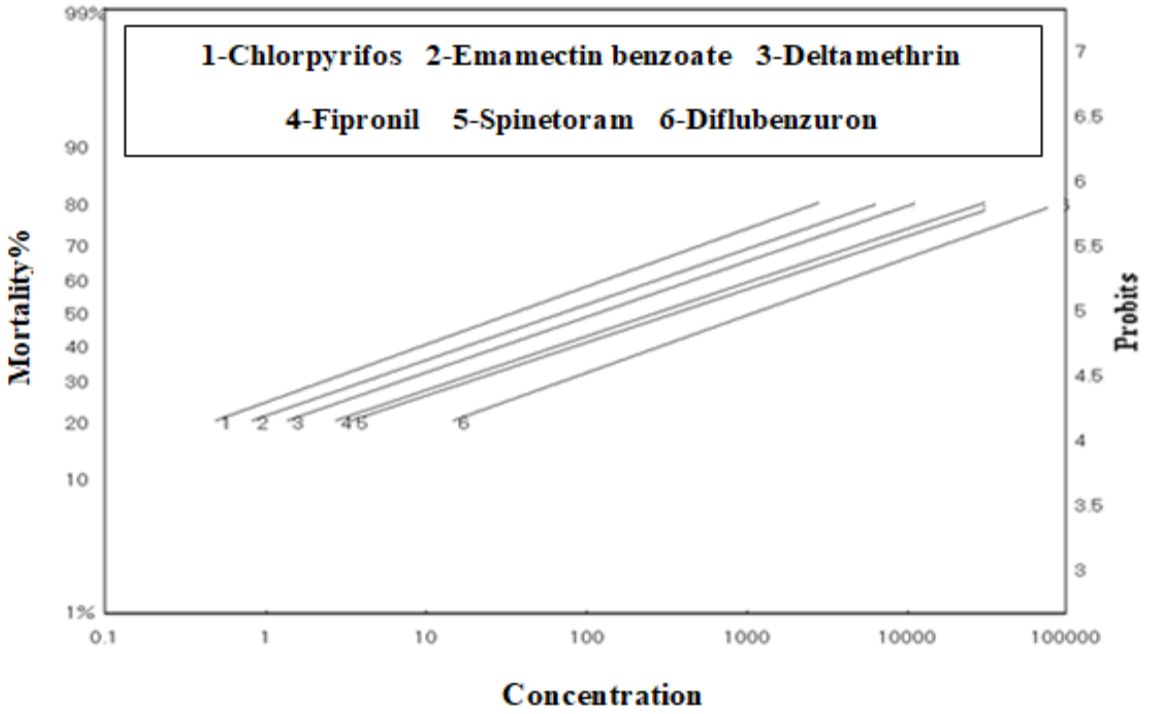
Table 3 and Fig.5 showed that the mean mortality rate of termite workers after treatment with concentrates of spore suspension of *M. anisopliae* and *B. bassiana* after 7 days. The fungi *M. ansiopliae* more effectiveness than *B. bassiana* which the mortality rates were 25% and 17.5% for *M. ansiopliae, B. bassiana,* respectively. After 14 days of treatments with increased period the result shows the mortality rates were 38.3% and 29.1% to *M. ansiopliae, B. bassiana* respectively. While, after 21 days of treatment, the results show the mortality rates were 55.8% and 48.3% to *M. ansiopliae, B. bassiana,* respectively. These results indicated that *M. anisopliae* was the most toxic against subterranean termite, *P. hypostoma* than *B. bassiana*. These results agree with Solaiman and Abd EL-Latif (2014) who found the mortality rates were 26, 84 and 100%, respectively during 7days of termite, *P. hypostoma* workers treatment with *M. anisopliae* spores. The mortality rate was the least in the first two days of treatment and increased rapidly in the following days. *M. anisopliae* at 10^8 spores/ml caused 57.5, 77 and 100% mortality among treated *C. formosanus* on day 7, 14 and 21, respectively.

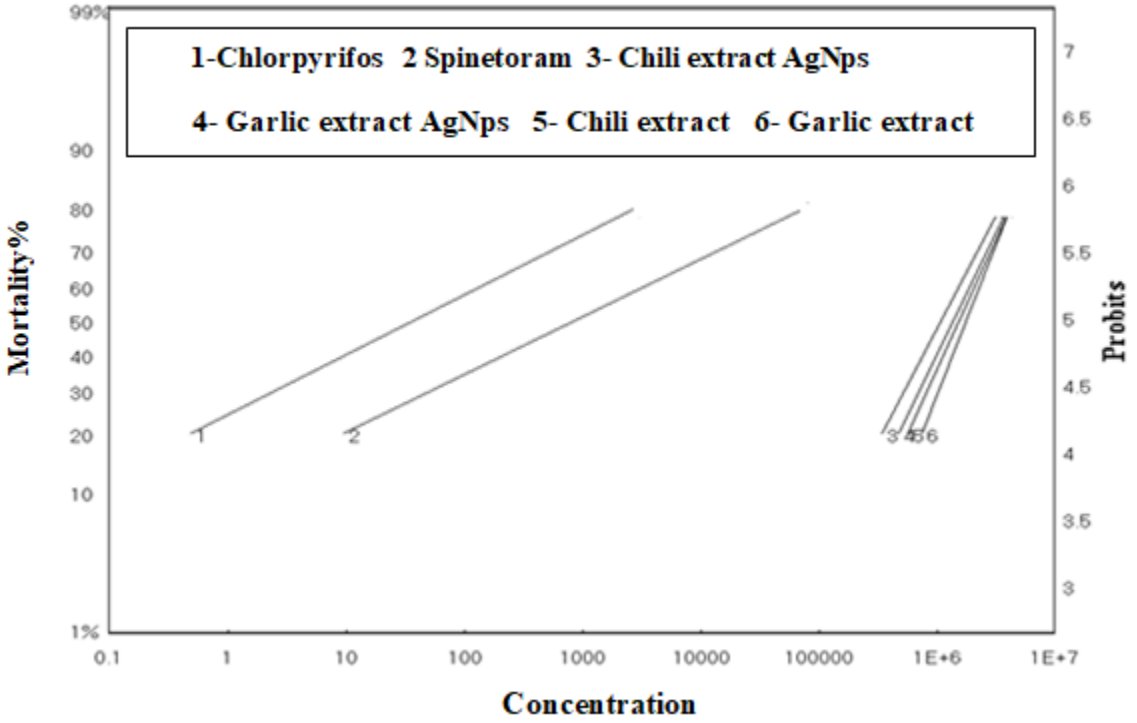
Table 2. Toxicity of tested insecticides, conventional and nano silver plant extract against *P. hypostoma*, using cardboard-dip bioassay after 24, 48 and 72 h exposure.

Insecticides, conventional and nano silver extracts	LC_{50} *ppm	Confidence limits of LC_{50}		χ^2	Slope ±SE	Toxicity index (T.I.)
		Lower	Upper			
After 24 h						
Chlorpyrifos	38.19 a	12.4	158.77	1.02	0.45 ±0.08	100
Deltamethrin	128.56 a	128.56	938.19	0.39	0.43 ±0.08	29.70
Diflubenzuron	1204.21 ab	1204.21	279970.1	0.17	0.44 ±0.09	3.17
Fipronil	299.41 a	299.41	3345.8	1.19	0.42 ±0.08	12.75
Imidacloprid	396.74 a	396.74	5449.3	1.08	0.41 ±0.08	9.62
Emamectin benzoate	77.06 a	23.60	425.75	0.94	0.43 ±0.08	49.55
Spinetoram	880.94 ab	880.94	17671.5	0.72	0.43 ±0.09	4.33
Chili extract AgNPs	51865.3 c	38644.2	68511	3.2	1.89±0.45	0.074
Chili extract	156120 d	103290	656610	3.76	1.92±0.58	0.024
Garlic extract AgNPs	110320d	78420.9	289560	1.01	1.69±0.49	0.035
Garlic extract	178219 d	115405.6	996877.9	2.43	2.25± 0.72	0.021
After 48 h						
Chlorpyrifos	8.67 a	2.47	31.18	1.70	0.44 ±0.083	100
Deltamethrin	37.69 a	11.86	164.42	0.35	0.44 ±0.083	23.01
Diflubenzuron	144.06 ab	51.51	634.93	1.18	0.53 ±0.094	6.02
Fipronil	42.48 a	14.05	175.79	0.47	0.45 ±0.084	20.41
Imidacloprid	67.39 a	24.09	264.21	1.13	0.50 ±0.088	12.87
Emamectin benzoate	22.25 a	7.20	81.86	0.49	0.45 ±0.082	38.98
Spinetoram	92.42 ab	32.35	398.10	1.43	0.50 ±0.089	9.38
Chili extract AgNPs	49097.3 c	32025.6	68614.6	3.58	1.48±0.42	0.018
Chili extract	84243.7 cd	62969.5	156768	0.69	1.71±0.47	0.010
Garlic extract AgNPs	59312.9cd	42428.8	90876.5	1.36	1.54±0.44	0.015
Garlic extract	105230 cd	76239.4	245769	1.74	1.75±0.49	0.0082
After 72 h						
Chlorpyrifos	0.30 a	0.03	1.07	0.36	0.46 ±0.084	100
Deltamethrin	2.02 a	0.44	6.34	0.14	0.44 ±0.083	15.22
Diflubenzuron	21.34 ab	7.40	70.57	2.51	0.53 ±0.083	1.44
Fipronil	3.67 a	0.92	11.51	0.71	0.44 ±0.082	8.39
Imidacloprid	4.87 ab	1.16	16.21	0.15	0.42 ±0.083	6.32
Emamectin benzoate	1.39 a	0.27	4.42	0.84	0.44 ±0.084	22.09
Spinetoram	9.3 ab	2.92	29.13	2.57	0.46 ±0.084	3.31
Chili extract AgNPs	17102.9 c	6229.2	25431.1	1.12	1.93±0.48	0.0018
Chili extract	35249.2 c	22085	46026.4	0.48	1.85±0.44	0.0009
Garlic extract AgNPs	22208.9cd	9154.2	31744.9	2.01	1.75±0.45	0.0014
Garlic extract	49065.4cd	36004.4	65134.4	2.4	1.9±0.46	0.0006

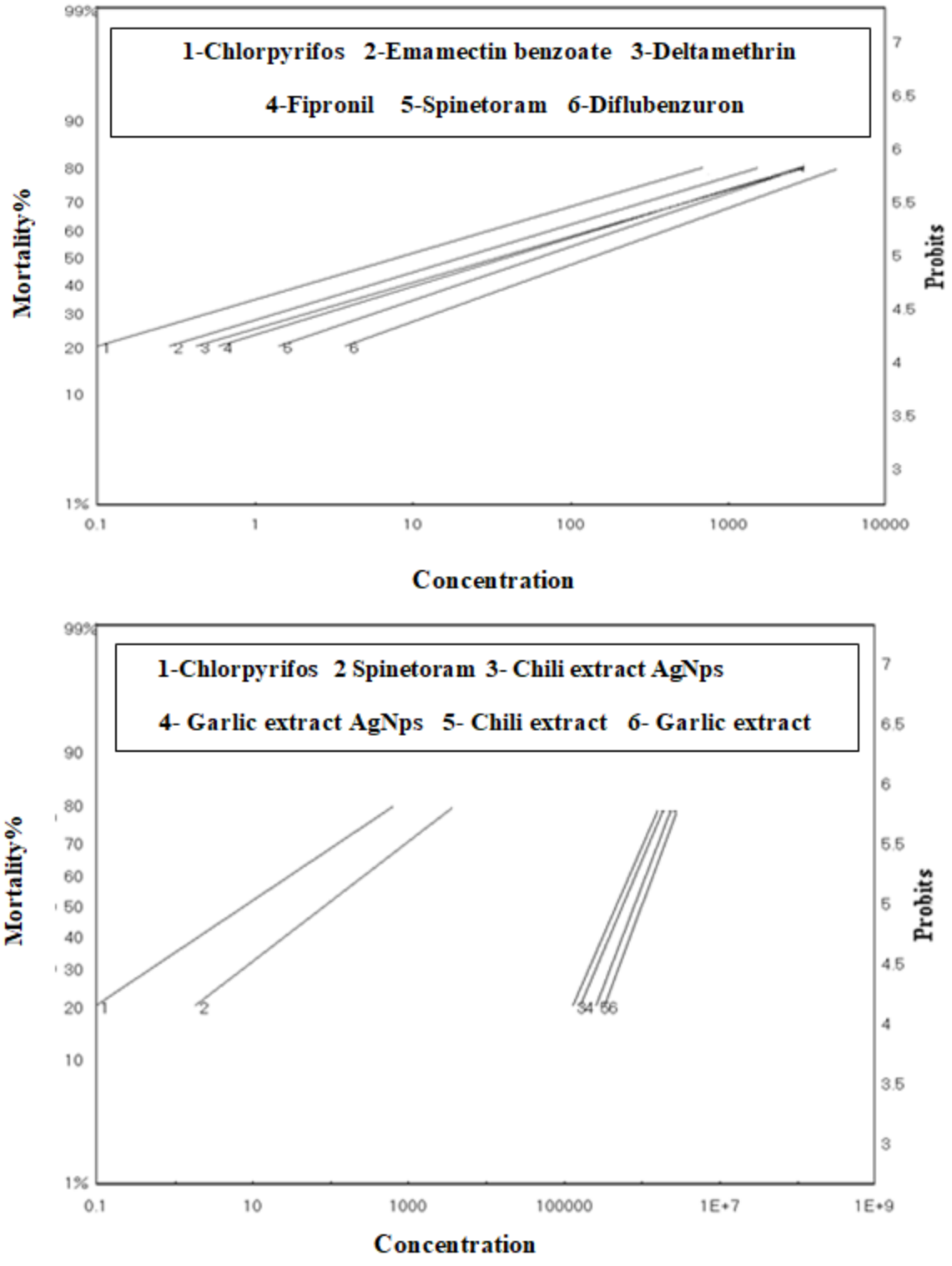
T.I. - Toxicity index compared with chlorpyrifos $\chi 2$ = Chi-square

LC_{50} values in same column with different letters are significantly different (95% FL did not overlap based of confidence limits values)

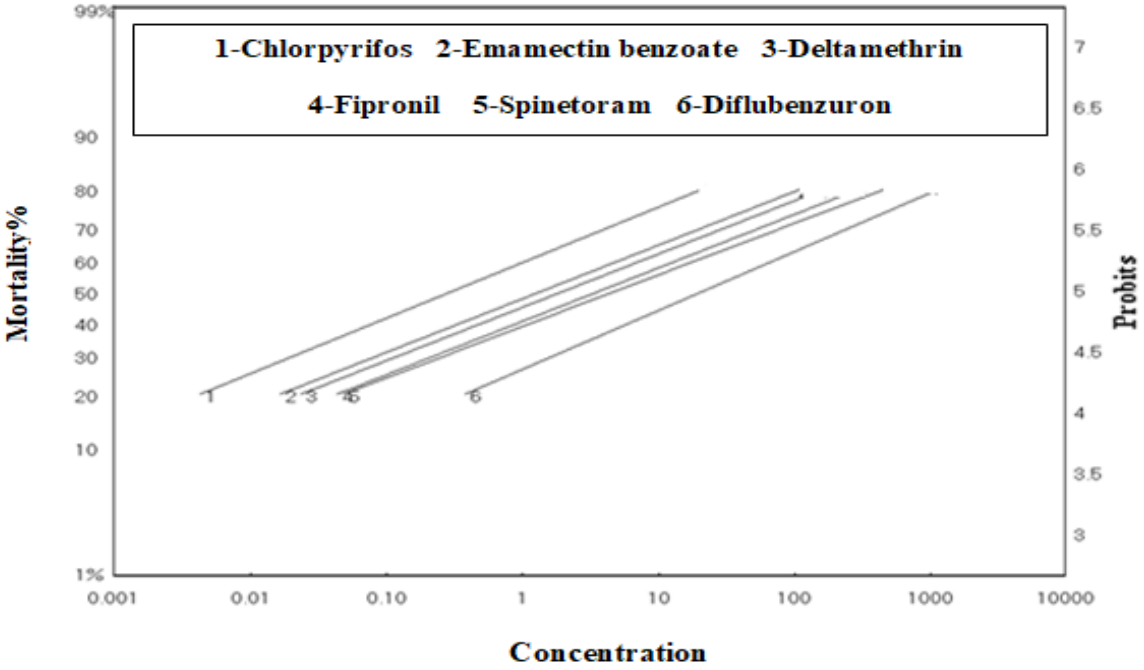


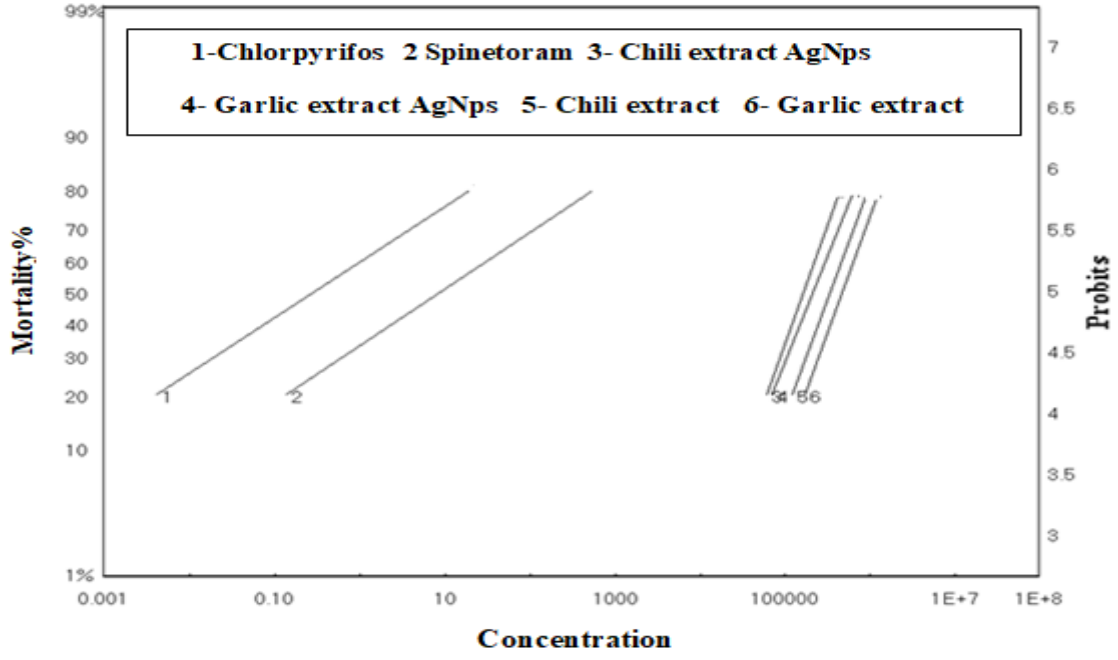


(A)-Toxicity lines of tested insecticides compare to chloropyrifos against *P. hypostoma* after 24 h.



(B)-Toxicity lines of tested insecticides compare to chloropyrifos against *P. hypostoma* after 48 h.





(C)-Toxicity lines of tested insecticides compare to chloropyrifos against *P. hypostoma* after 72 h.

Figs 4. Toxicity of tested insecticides compare to chloropyrifos against *P. hypostoma* after 24, 48 and 72 h**.**

The present study supported by Singha *et al.* (2011) who reported that *M. anisopliae* was achieved higher mortality compared with *B. bassiana* when used application a topical applied on tea termite worker, *Microtermes obesi*. Moreover, *M. anisopliae* the mortality rate was 43.28-72.24% after 7 days from treatment against termite infesting tea Hoque *et al.*,

(2016). (Soliman *et al,* 2019) show that the fungi *M.ansiopliae* more effectiveness than *B.bassiana* and *A .niger* which the mortality rates were 22.5%, 15.0% and 13.3% to *M.ansiopliae, B.bassiana* and *A. niger,* respectively. The potential use of *M. anisopliae* as a biological control agent against subterranean termites is being challenged, because the disease remained at an enzootic level in laboratory sand arenas and the fungus showed a reduced survival rate in the presence of termites Chouvenc *et al.* (2008). The mode of action of an entomopathogen is difficult because it is possible for a small amount of vaccine to spread all over the nest before discovery, resulting in an epidemical condition (Kramm *et al.*, 1982). Commercial formulations of entomopathogenic fungi are successfully applied as an alternative to chemical agents in control of agricultural pests such as like *B. bassiana* and *M. anisopliae.* Entomopathogenic fungi infest the host insects via digestion, respiration and through integument. In infestation from integument, which is one of the most common infestation methods, fungi grow hyphae to penetrate epicuticle and progresses into hypodermis to achieve the infestation. Anamorphic fungi like *B. bassiana* and *M. anisopliae* primarily propagates as blastospores rather than hyphal development and these blastospores invade the vital organs by dispersing across the insect body via circulation of hemolymph within body cavity and eventually result in death of insect by clogging the circulatory system (Altinok *et al.,* 2019).

Why the tested entomopathogenic fungi have low effect, although, the weather in cardboard should be optimum for growing of these fungi. We expected high efficacy on the field testing especially on termites' tunnels. Further, studies should be applied in these points. Moreover, the mixture of the chlorpyrifos with the entomopathogenic fungi or plant extracts should be applied to decrease the hazard effects of the organophosphorus insecticide on the environment. Also, the integrated pest management (IPM) should be designed to give chance to every useful component to share in control the termites. The termite's dynamics and their insecticide susceptibility studies should be continuously recorded every year with reference the world climate change.

Table 3. Mean mortality (±SE) virulence of two pathogenic fungi, *M. anisopliae* and *B. bassiana* against *P. hypostoma* after 7, 14, and 21days.

Entomopathogenic fungi	(Mean ±SE) Mortality%*
	After 7 day
B. bassiana	17.5±5.09 a
M. anisopliae	25±5.71 a
	After 14 day
B. bassiana	29.1±6.56 a
M. anisopliae	38.3±7.37 a
	After 21 day
B. bassiana	47.5±8.68 a
M. anisopliae	55.8±8.91 a

*Each mean mortality rate (%) value represents mean of 12 replicates from 4 treatments.

**Means in same column with different letters are significantly different (P > 0.05) according to Duncan's Multiple Range Test (DMRT)

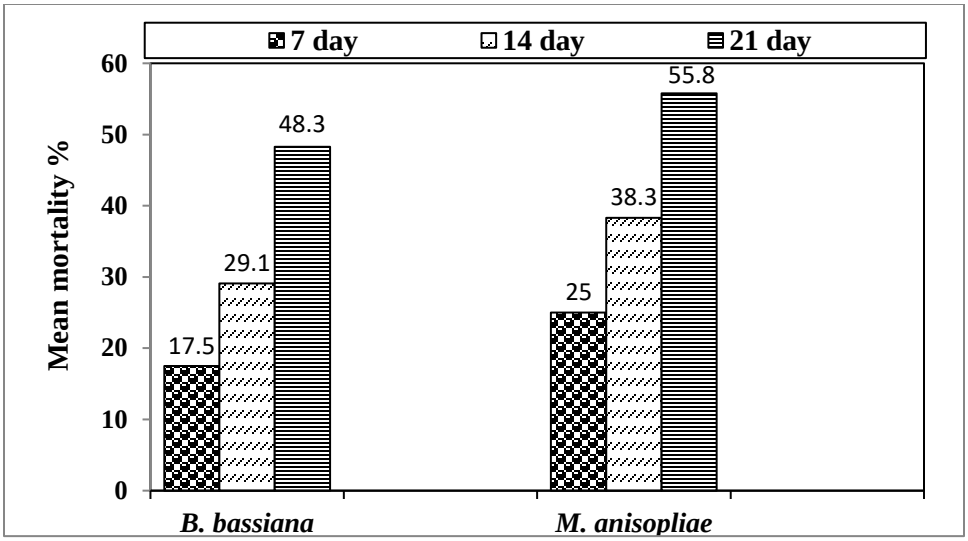


Fig 5. Accumulative mortality of entomopathogenic fungi against *P. hypostoma* after 7, 14, and 21days**.**

4. Conclusion

The results introduce several new effective compounds represented from different groups which can used in integrated pest management programs of *P. hypostoma*. Biopesticide and silver nanoparticles extracts are promised compounds and had low toxicities in mammals and human.

Authors' Contributions

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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