UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v7i04.6648
Uni. Sindh. J. Anim. Sci., vol. 7(4); 61-68, 2023
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

COMPARATIVE EFFICACY OF BOTANICAL EXTRACTS ALONG WITH THEIR BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES AGAINST HOUSE FLY *MUSCA DOMESTICA* L. (DIPTERA: MUSCIDAE)

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ARTICLE INFORMATION

Article History:
Received: 29th July 2023
Accepted: 05th December 2023
Published online: 31st December 2023

Author's contribution:
RMMA conducted research plan and wrote manuscript, MHB supervised the research, MIS contributed to data analysis, SUH helped in data interpretation, SK performed statistical analysis, HS contributed to experimental design, AB helped in manuscript preparation, KL and KW reviewed the article.

Key words:
Musca domestica, Botanicals, Zinc oxide nanoparticles, Azadirachta indica, Citrus limon and Mortality.

ABSTRACT

The current study was conducted in 2023 at the Department of Entomology, University of Agriculture, Faisalabad, in a Completely Randomized Design with three replications. The objective of this research was to determine the efficiency of botanical extracts and their green synthesized zinc oxide nanoparticles against *Musca domestica*. Results revealed that at 50% concentration, *Azadirachta indica* extracts exhibited the highest mortality on larvae (51%), pupae (49%) and adults (53%), respectively, while at same concentration *Citrus limon* extracts exhibited the highest mortality on larvae (41%), pupae (45%) and adults (44%), respectively. Similarly, at 400ppm, ZnO Nps of *A. indica* showed 64, 57 and 67% mortality on larvae, pupae, and adults, respectively. While ZnO Nps of *C. limon* showed mortality on larvae (50%), pupae (54%) and adults (55%), respectively. The findings showed that *A. indica* extract at 50% concentration was more toxic to *M. domestica* as compared to *C. limon* extract. Additionally, *A. indica* ZnO Nps at 400ppm concentration were highly toxic to *M. domestica* as compared to *C. limon* ZnO Nps extract.

1. INTRODUCTION

The House fly, *Musca domestica* L. (Diptera: Muscidae) is a global health pest that poses a significant threat to both humans and animals by vectoring a variety of contagious diseases (Baker *et al.*, 2020). The house fly spread some of its microbial content whenever it lands on human drinks or food by depositing it in saliva or feces (Park *et al.*, 2019). Flies pick up pathogens via sewage, waste and other unsanitary sources (Abbas *et al.*, 2013). The house fly has a tendency for dispersing illnesses investigation has also revealed that house flies contain a diverse range of pathogens and viruses (Bahrndorff *et al.*, 2017).

More than 100 pathogens that are dangerous to humans can be carried with house flies, including bacteria like *Yersinia pseudotuberculosis*, *Vibrio cholerae*, *Helicobactor pylor*, *Campylobacter jejuni*, *Salmonella enteritidis* and *Escherichia coli (*Khamesipour *et al.*, 2018). It was discovered that house flies are important avian flu virus transmitters. Which poses a threat to humans, poultry and the global livestock industry (Wanaratana *et al.*, 2013). According to reports, the seasonal abundance of house flies in developing countries like Pakistan is a contributing factor to the spread of digestive disorders like diarrhea in urban and rural regions (Khan *et al.*, 2013).

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Insecticides play a significant role in suppressing this destructive pest. Many chemicals are used to control house flies and that is considered as rapid control of pest population (Walsh *et al.,* 2022). Unknowingly implemented of insecticides at greater quantities in lawns, gardens and houses that seriously jeopardizes the quality of the environment, living things and food. In mammals by inhalation, insecticides can cause oxidative stress, liver toxicity, kidney damage, hepatotoxicity, teratogenicity, carcinogenicity, mutagenicity, as well as neurodegeneration (Muhoro & Farkas, 2021; South *et al.,* 2020). Pesticide usage is less successful due to several variables, including insecticide resistance, rising insecticide prices and insecticide toxicity for other beneficial insects (Scott, 2017).

Traditional approaches of managing insect pests may be replaced with botanical pesticides, which are abundant in bioactive chemicals (Gusmao *et al.,* 2013). They are effective against specific target species, are non-toxic to mammals and humans, and useful in integrated pest management (Ngegba *et al.,* 2022). It has been demonstrated that the chemical components of biopesticides have insecticidal and repellant properties (Nwanade *et al.,* 2020). Natural plant-based insecticides are a promising alternative to synthetic chemical pesticides for the management of insect pests (Ahmed *et al.,* 2021). The neem tree, *Azadirachta indica*, contains a variety of chemical materials that can be used as insecticides and has the potential to control insect pests (Boeke *et al.,* 2004). Azadirachtin is very well known for its capacity to suppress phytogenic pest growth. It prevents pests of various orders from feeding and growing (Pavela, 2007). Because of their several mechanisms of action on insects, neem compounds have little toxicity to birds, fishes and humans and are less likely to cause resistance. Neem has been used in a variety of mosquito control efforts due to its effectiveness in controlling agricultural pests, environment protection and public acceptance of its products (Acharya *et al.,* 2017). Lemon, also known as *Citrus limon*, has been investigated for its potential as a biopesticide because of its inherent ability to deter or suppress pests (Morya *et al.,* 2010). Numerous substances found in lemons have been demonstrated to have insecticidal and repellant effects. Citronellal, citronellol and limonoids are some of the active substances present in lemons that contribute to their biopesticide activities (Aslam *et al.,* 2011). These substances are helpful in pest management methods since they may deter and even kill some pests (Mossa, 2016).

Due to their antibacterial and pesticide properties, nanoparticles have received a lot of interest nowadays (Kalpana *et al.,* 2018). Additionally, compared to their synthetic equivalents, these environmentally friendly nanoparticles are said to be more effective pesticides, cheaper, biodegradable and safer for both humans and the environment (Murugan *et al.,* 2016). For the creation of nanoparticles, a variety of botanicals with different reductive groups can serve as reducing and protecting agents. Harmful chemicals are not used in the synthesis process, using botanicals to create nanoparticles has many advantages (Borase *et al.,* 2014). On the *M. domestica*, green synthesized nanoparticle has been found to exhibit bio insecticidal properties (Al-Nasser *et al.,* 2022). The experiments showed that ZnO Nps have an exceptionally powerful antimicrobial action at incredibly low gram-negative and gram-positive bacterial concentrations, suggesting a stronger antibacterial than chemically produced ZnO Nps (El-Ghwas *et al.,* 2022). The objective of this research is to investigate the comparative efficacy of botanical extracts *A. indica* and *C. limon*, along with their biosynthesized zinc oxide nanoparticles against larvae, pupae and adults of *M. domestica*.

2. MATERIALS AND METHODS

Study area

This study was conducted in the Department of Entomology at the University of Agriculture in Faisalabad.

Collection of house fly

Adult houseflies were collected by using the sweep net from garbage, livestock farms and chicken shops at various locations in Faisalabad, Punjab, Pakistan.

Laboratory rearing

The collected house flies were released in plastic cages having size 40 cm × 45 cm × 45 cm. A cotton wick moistened in the water along with powdered milk and sugar mixed (1:1 w/v) was served as food source in a separate petri dish for adults. Larvae was reared on artificial diet that contains powdered milk, sugar, yeast, grass meal and wheat bran in the following proportions: 0.3:0.3:1:2:4 w/v, respectively (Francuski *et al.,* 2020). House flies were kept under controlled laboratory environment of 26±28°C, R.H 60–70%, and a photoperiod of (14:10 LD). To determine the exact age of adults, emerged adults from pupae were transferred to separate containers.

Collection of leaves and peel

Neem leaves that were fully developed and fresh as well as lemon peel were taken from the University of Agriculture, Faisalabad botanical garden.

Preparation of extract

Fresh neem leaves and lemon peel were washed, dried in the shade for eight days and then chopped. The chopped materials were ground to a fine powder using a grinder and sieved through a 40-mesh size. Then 5g of each powder was taken, mixed in 500ml of distilled water and shaken on a shaker. The mixture was then heated at 50°C for 1 hour at 40 rpm in a rotary evaporator, separately for both samples. The resulting solutions were purified using Whatman no. 1 filter paper to obtain pure liquid extracts, which were collected in a bottom flask and stored at 4°C.

Biosynthesis of ZnO nanoparticles

A magnetic stirrer was used to heat 100ml of prepared extracts and 0.81g zinc oxide to make a 10mM solution in 1000ml of distilled water at room temperature. It was covered with aluminum foil to prevent hydrolysis when exposed to light. The change in color indicated the formation of ZnO nanoparticles. The solution was kept in incubator at 28°C. Then a chemical reaction occurred after the couple of days and then nanoparticles were formed in liquid state.

UV spectrophotometry of ZnO NPs

UV spectrophotometry was used to clarify nanoparticles.

Larvicidal bioassay

The relative efficacy of plant extracts and ZnO Nps against *M. domestica* larvae was evaluated using the larvae dipping method. Different concentration of ZnO Nps (400, 300, 200 and 100 ppm) and botanical extract (50, 25, 12.50 and 6.25%) was made from stock solution. Twenty larvae of the third instar were carefully immersed for 30 seconds within every dosage before being returned to the rearing medium. For the control, larvae were immersed in distilled water. The experiment consists of three replications. After (24, 48, 72 h) of exposure, susceptible larvae were counted by being touched with a soft camel hairbrush.

Pupicidal bioassay

The topical application method was used to evaluate the pupicidal activities of botanical extracts and ZnO Nanoparticles. Each concentration of an individual botanical extracts and ZnO Nanoparticles was administered to twenty pupae that are one day old with the help of micropipette. After 7 days of exposure, un-emerged adults from were counted. The experiment was replicated three times.

Adulticidal bioassay

Twenty 3-4 days old *M. domestica* were given each dosage of botanical extract and ZnO Nps on the thoracic notum for adulticidal toxicity studies. Only distilled water was used to treat *M. domestica* in the control group. Following treatment, *M. domestica* was moved to enclosures for rearing and given dry sugar, milk powder and cotton swabs wet in water. The experiment consists of three replications. The number of dead estimates were made (24, 48, 72 h) after the exposure.

Statistical analysis

All collected results were subjected to statistical analysis. The data collected were analyzed using a one-way analysis of variance (ANOVA) and means were compared using Tukey's HSD test.

3. RESULTS AND DISCUSSION

Mortality is caused by botanicals and biosynthesized zinc oxide nanoparticles on the larval stage of Musca domestica.

All tested botanicals and biosynthesized zinc oxide nanoparticles caused significant mortality of house fly larvae after 72 hours of application as shown in Table 1 and 2. The *Azadirachta indica* mediated ZnO Nps was statistically highly effective with mortality of 30.67% followed by *Citrus limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 27.33, 24.67 and 20.00% respectively, after 24 hours of application. The *A. indica* mediated ZnO Nps was again significantly more efficient with mortality of 50% than the *C. limon* mediated ZnO Nps, *A. indica* and *C. Limon* was 40.33, 37.00 and 33.67% respectively, after 48 hours of application. However, after 72 hours application, *A. indica* mediated ZnO Nps caused the highest mortality 63.67% followed by *C. limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 50.00, 46.33 and 40.67% respectively which were also significantly different from each other. The results demonstrated that *A. indica* extract was more toxic to *M. domestica* larvae as compared to *C. limon* extract at a 50% concentration. In contrast to *C. limon* ZnO Nps extract, *A. indica* ZnO Nps at 400 ppm concentration was highly lethal to *M. domestica* larvae.

Mortality is caused by botanicals and biosynthesized zinc oxide nanoparticles on the pupal stage of Musca domestica.

All tested botanicals and biosynthesized zinc oxide nanoparticles caused significant mortality of house fly pupae after 7 days of application as shown in Table 3 and 4. The *Azadirachta indica* mediated ZnO Nps was statistically highly toxic with mortality of 56.33% followed by *Citrus limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 53.33, 48.33 and 45.00% respectively, after 7 days of treatment exposure. The results showed that *A. indica* extract was more toxic to *M. domestica* pupae as compared to *C. limon* extract at a 50% concentration. In contrast to *C. limon* ZnO Nps

extract, *A. indica* ZnO Nps at 400 ppm concentration was highly lethal to *M. domestica* pupae.

Mortality caused by botanicals and biosynthesized zinc oxide nanoparticles on the adult stage of Musca domestica.

All tested botanicals and biosynthesized zinc oxide nanoparticles caused significant mortality of house fly adult stage after 72 hours of application as shown in Table 5 and 6. The *Azadirachta indica* mediated ZnO Nps was statistically highly effective with mortality of 40.00% followed by *Citrus limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 35.67, 32.33 and 26.33% respectively, after 24 hours of application. The *A. indica* mediated ZnO Nps was again significantly more efficient with mortality of 52.33% than the *C. limon* mediated ZnO Nps, *A. indica* and *C. Limon* was 43.00, 40.67 and 32.33% respectively, after 48 hours of application. However, after 72 hours application, *A. indica* mediated ZnO Nps caused the highest mortality 66.67% followed by *C. limon* mediated ZnO Nps, *A. indica* and *C. Limon* with mortality of 55.00, 52.67 and 43.33% respectively which were also significantly different from each other. The botanicals and biosynthesized zinc oxide nanoparticles after 72 hours of treatments application showed gradual reduction in percentage mortality of the house fly larvae as compared to 24 and 48 hours after treatment exposure. The findings showed that *A. indica* extract at 50% concentration was more toxic to *M. domestica* adults as compared to *C. limon* extract. Additionally, *A. indica* ZnO Nps at 400ppm concentration were highly toxic to *M. domestica* adult stage as compared to *C. limon* ZnO Nps extract.

The current findings are in line with the findings of Aisvarya *et al.*, (2023) reported that silica nanoparticles showed 73% mortality of *Sitophilus oryzae* at the dose of 9 mg after 4 days. Similar observation was also recorded by Al-Azzazy and Ghani (2023) determined the effect of copper nanoparticles against *Phyllocoptruta oleivora*. Results showed that 86% mortality of *P. oleivora* at the dose of 320 ppm after one week. Our results were similar to Ayinde *et al.*, (2020) investigated the larvicidal activity of *Azadirachta indica* seed oil on *Anopheles gambiae.* A larval mortality rate of 100.0% was observed in *Anopheles gambiae* within a three-day period at a concentration of 500 ppm. Our results were similar to Gogate *et al.*, (2018) showed that biosynthesized ZnO Nps revealed 83% toxicity in *Corcyra cephalonica*. Our findings were similar to Ghidan *et al.*, (2017), who stated that ZnO nanoparticles, facilitated by *Punica granatum*, resulted in a mortality rate of 75.5% for the first and second nymphal instar of *Myzus persicae* after 24 hours at a concentration of 8000 µg/ml. Our results were consistent with the findings of Buhroo *et al.*, (2017), who observed that ZnO nanoparticles induced 100% mortality in 4th instar larvae of *Mythimna separate* at a concentration of 500 ppm under laboratory conditions. Additionally, concentrations of 400 ppm, 300 ppm, and 200 ppm resulted in mortality rates of 93%, 83%, and 46% respectively for the 4th instar larvae of *M. separate*.

4. CONCLUSION

These findings highlight the high efficacy of zinc oxide nanoparticles in managing the population of *Musca domestica* while posing no risks to human health and the environment, making them a promising tool for integrated pest management.

5. ACKNOWLEDGEMENTS

Author is extremely thankful to Dr. Muhammad Hamid Bashir, Associate Professor, Department of Entomology, University of Agriculture, Faisalabad, for providing invaluable guidance throughout this research.

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Mean percent mortality (M±SE) of *Musca domestica* larvae at 24, 48 and 72 hours after the application of botanicals in a laboratory test.

Sr. No.	Treatments	Concentration (%)	24 h	48 h	72 h
1	*Azadirachta indica*	6.25	1.23±1.05^{b}	10.00±3.33^{b}	16.67±3.33^{c}
		12.50	6.67±2.05^{ab}	13.33±2.28^{bc}	20.00±2.33^{c}
		25	16.67±2.05^{ab}	26.67±3.32^{ab}	40.00±2.45^{b}
		50	24.67±3.33^{a}	37.00±2.46^{a}	50.67±3.33^{a}
2	*Citrus limon*	6.25	6.67±1.20^{ab}	13.33±1.12^{c}	16.67±3.33^{bc}
		12.50	10.00±2.50^{ab}	13.33±2.85^{ab}	20.00±2.85^{bc}
		25	13.33±3.33^{ab}	16.67±4.08^{ab}	30.00±3.33^{bc}
		50	20.00±4.08^{a}	33.67±3.33^{a}	40.67±3.04^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error

Table 2. Mean percent mortality (M±SE) of *Musca domestica* larvae at 24, 48 and 72 hours after the application of biosynthesized ZnO Nps in a laboratory test.

Sr. No.	Treatments	Concentration (ppm)	24 h	48 h	72 h
1	*Azadirachta indica* ZnO Nps	100	6.67±3.04^{b}	13.33±2.20^{cd}	16.67±3.33^{cd}
		200	13.33±2.08^{ab}	20.00±3.33^{bc}	36.67±2.45^{ab}
		300	20.00±1.23^{ab}	30.00±2.78^{b}	40.00±2.45^{ab}
		400	30.67±3.33^{a}	50.00±3.33^{a}	63.67±3.33^{a}
2		100	6.67±2.76^{b}	16.67±1.45^{ab}	20.00±3.12^{bc}

	Citrus limon ZnO Nps	200	10.00±1.46^{ab}	20.00±2.38^{ab}	30.33±3.33^{ab}
		300	16.67±2.23^{ab}	23.33±3.33^{ab}	38.33±2.45^{ab}
		400	27.33±3.33^{a}	40.33±3.48^{a}	50.00±3.33^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error
ZnO Nps: Zinc oxide nanoparticles
Ppm: Part per million

Table 3. Mean percent mortality (M±SE) of *Musca domestica* pupae after 7 days of application of botanicals in a laboratory test.

Sr. No.	Treatments	Concentration (%)	7 days
1	*Azadirachta indica*	6.25	14.33±3.33^{b}
		12.50	23.00±2.33^{b}
		25	36.00±2.58^{ab}
		50	48.33±3.33^{a}
2	*Citrus limon*	6.25	13.33±3.33^{bc}
		12.50	20.67±1.33^{ab}
		25	32.33±1.58^{ab}
		50	45.00±4.38^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error

Table 3. Mean percent mortality (M±SE)) of *Musca domestica* pupae after 7 days of application of botanicals in a laboratory test.

Sr. No.	Treatments	Concentration (ppm)	7 days
1	*Azadirachta indica* ZnO Nps	100	19.33±1.33^{c}
		200	25.33±2.33^{bc}
		300	40.67±2.58^{b}
		400	56.33±3.33^{a}
2	*Citrus limon* ZnO Nps	100	16.67±3.33^{a}
		200	22..00±3.21^{bc}
		300	36.33±1.49^{b}
		400	53.33±3.33^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error
ZnO Nps: Zinc oxide nanoparticles
Ppm: Part per million

Table 5. Mean percent mortality (M±SE) of *Musca domestica* adults at 24, 48 and 72 hours after the application of botanicals in a laboratory test.

Sr. No.	Treatments	Concentration (%)	24 h	48 h	72 h
1	*Azadirachta indica*	6.25	13.33±3.04^{bc}	16.67±3.33^{b}	20.00±2.33^{bc}
		12.50	18.67±3.46^{b}	22.00±3.12^{b}	28.33±3.33^{b}
		25	23.33±0.33^{ab}	30.33±4.32^{ab}	36.67±2.45^{ab}
		50	32.33±3.33^{a}	40.67±3.33^{a}	52.67±4.43^{a}
2	*Citrus limon*	6.25	10.00±0.21^{c}	13.67±1.12^{c}	20.00±2.50^{bc}
		12.50	13.33±3.33^{bc}	19.00±2.61^{ab}	25.35±3.33^{abc}
		25	20.00±3.58^{ab}	23.33±3.23^{ab}	33.33±4.00^{ab}
		50	26.33±3.67^{a}	32.33±3.67^{a}	43.33±3.04^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error

Table 6. Mean percent mortality (M±SE) of *Musca domestica* adults at 24, 48 and 72 hours after the application of biosynthesized ZnO Nps in a laboratory test.

Sr. No.	Treatments	Concentration (ppm)	24 h	48 h	72 h
1	*Azadirachta indica* ZnO Nps	100	16.37±0.67^{b}	23.33±1.02^{d}	26.67±3.04^{c}
		200	20.00±1.06^{bc}	26.67±1.56^{b}	38.33±4.32^{bc}
		300	29.67±2.23^{ab}	36.67±2.78^{ab}	50.00±2.45^{ab}
		400	40.00±3.33^{a}	52.33±3.33^{a}	66.67±3.33^{a}
2	*Citrus limon* ZnO Nps	100	14.33±3.04^{ab}	18.67±1.70^{bc}	23.33±4.08^{bc}
		200	21.33±2.46^{ab}	25.33±2.38^{abc}	32.67±3.33^{bc}
		300	27.67±3.23^{a}	33.67±3.12^{ab}	42.00±2.45^{ab}
		400	35.67±4.13^{a}	43.00±4.02^{a}	55.00±3.33^{a}

Means within a column not sharing a common letter are significantly different at $p < 0.05$ using Tukey's test.
M±SE: Mean ± Standard error
ZnO Nps: Zinc oxide nanoparticles
Ppm: Part per million