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EVALUATING THE TOXICITY OF PIPERINE, AZADIRACHTIN, AND QUERCETIN AGAINST *SOLENOPSIS INVICTA* BUREN (HYMENOPTERA: FORMICIDAE) WORKERS VIA FEEDING BIOASSAY UNDER CONTROLLED CONDITION

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A B S T R A C T

Exploration of effective management technique against *Solenopsis invicta* Buren (Hymenoptera: Formicidae) has gained the great concern of researchers because of its well-known negative impacts on public health, economic development and native biodiversity. Among various control measures, use of botanical extracts and essential oils are being highly focused because of eco-friendly in nature. Current study explains the effectiveness of three bioactive compounds including Piperine, Azadirachtin, Quercetin and all of their possible combinations against *S. invicta* workers. Sugar water (20% w/w) was used as solvent for these treatments. Data of mortality were recorded after every 24, 48 and 72 hours. Results revealed that all the treatments caused satisfied mortality in exposed fire ant workers. The mortality was directly proportional to concentrations and time of exposure in all treatments. Among all the treatments, Piperine and its combinations caused highest mortality of fire ant worker. This study will be more useful to future work regarding chemical ecology of social ants. The results of research presented great potential of these biopesticides against *S. invicta*.

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INTRODUCTION

The red imported fire ant (RIFA), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is considered a native social pest of South America and most notorious social pest in China (Jaleel et al., 2021a; Jaleel et al., 2021b; Lei et al., 2021; Vinson, 1997). Their mounds are frequently seen in patches with varying densities; 600 mounds per hectare have been seen (Lei et al., 2019). Fire ants usually called “super pest” because of voracious impact on the social life of humans (Liu et al., 2021; Rashied et al., 2023).

Workers of *S. invicta* are oil lovers and mostly feed on sugar foods (Jaleel et al., 2021a; Wang et al., 2018; Howard and Tschinkel, 1980), nonetheless, were impacted by numerous biotic and abiotic elements (Lei et al., 2021; Bockoven et al., 2015; Vogt et al., 2003). Fire ants workers prefer liquid food, such as oil and sugar solution, as compared to solid food (Cassill and Tschinkel, 1999; Jaleel et al., 2021b; Wen et al., 2021).

Numerous studies reveal that insecticides are most prominent control method for S. Invicta (Goddard et al., 2002; Pereira et al., 2005; Sakamoto and Goka, 2021). One of limiting factor of insecticides is non-target toxicity and lingering residues, when pesticides were used to control red imported fire ants. Pesticides have detrimental effects on both human and environmental health. However, alternate of pesticides are botanical or biopesticides e.g., plant essential oils are less hazardous to the environment and humans. Due to their remarkable insecticidal and repellent qualities, they can be a great source of pesticides (Xing et al., 2023). Botanicals are more reliable, readily biodegradable, and have less risk of resistance development in red imported fire ants (Chen and Oi, 2020; Xing et al., 2023; Xie et al., 2020). Preparation of botanical pesticides are inexpensive as compared to synthetic insecticides (Akinbode et al., 2014; Guleria and Tiku, 2009; Bhandari et al., 2022). Generally speaking, botanicals are specialized and have little influence on the survival of natural adversaries (Grant et al., 2010; Giunti et al., 2022; Rizvi et al., 2019). Therefore, in the Integrated Pest Management (IPM) program, use of botanical pesticides are more effective control measures against social pests. It has been discovered that some phenolic chemicals are poisonous to insects e.g., red imported fire ants (Tian and Zhang, 2023). Potential of a sweet flag composition for treating the surface of various packaging materials toward off the social pests (Abhijith et al., 2019).

One of the most significant active ingredients that has been employed as a deterrent and poison against various pests is azadirachtin (Schmutterer, 1990; Naumann and Isman, 1995; Jaleel et al., 2020). Leaves powder of *Azadirachta indica* A. Juss (neem, nimtree, or Indian, and lilac) shows promising results for preventing ants (Korgaonkar et al., 2020). Neem extracts are best alternatives for the control of Dipteran pests (Ilyas et al., 2017; Jaleel et al., 2020). But no evidence was recorded against *S. invicta.*

One of the most significant fragrant spices and medicines has to be *Piper nigrum* L (Piperales: Piperaceae) (Damanhouri and Ahmad, 2014; Meghwal and Goswami, 2013; Takooree et al., 2019; Su and Horvat, 1981; Ahmad et al., 2012). Extracts and essential oils of *P. nigrum* have recently been employed as a deterrent and toxicant for number of pests in order Hymenoptera, Lepidoptera, Coleoptera, and Diptera (Jaleel et al., 2020; Freeborn and Wymore, 1929; Scott and McKibben, 1978). The *P. nigrum* was found best repellant against dipteran pests (Jaleel et al., 2020). However, no records were found against *S. invicta* workers.

Quercetin is a phenolic component found in a number of plants like apples, berries, brassica vegetables, capers, grapes, onions, spring onions, tea, and tomatoes, as well as in many seeds, nuts, flowers, bark, and leaves (David et al., 2016; Anjaneyulu and Chopra, 2003). Many insects from Lepidoptera (*Helicoverpa armigera*: Lepidoptera: Noctuidae), Diptera (*Bactrocera cucurbitae***:** Diptera: Tephritidae) and Hymenoptera (*Apis mellifera*: Hymenoptera**:** Apidae) are poisoned and repelled by quercetin (Ahmad and Pardini, 1990; Sharma and Sohal, 2013; Hidayatulfathi et al., 2017; Jaleel et al., 2020; Riddick, 2021). But there is no previous record of *S. invicta*.

These botanicals, *A. indica, P. nigrum,* and quercetin have not yet been tested against red imported fire ant workers, as far as the authors are aware. Therefore, the goal of this study was to assess the effectiveness of two botanicals components piperine, and one phenolic compound e.g., quercetin (95%) compound were used against *S. invicta* workers, while keeping in mind the significance of newly developing invasive pest and their safe control techniques.

MATERIALS AND METHODS

Ants' colonies

Solenopsis invicta Buren colonies were procured from South China Agricultural University's experimental area and raised in plastic containers (45 × 35 × 10 cm^3). Water was added to the plastic boxes until all ants floated, separating them from the soil. Live insects (*Tenebrio molitor* L.: Coleoptera: Tenebrionidae) and 10% honey were used to feed the ants. Water provided in a test tube (25×200 mm^2) that was blocked with cotton and only partially filled with water. Plastic container (25 × 17 × 8 cm^3) used for rearing the red imported fire ants acted as an artificial nest. By weighing the colonies, we were able to estimate their populations (g). We discovered that 1,000 workers weighed roughly 1 g. The CO_2 was used to put each colony to sleep for 10 seconds. Then, using delicate forceps, the workers were collected and by taking off the nest's cover, the precise number of queens was determined. Fire ant colonies in our experiment contained 10,000 workers, more than 10 queens, and more broods. Each tray of interior edge was painted with Fluon to prevent ants from escaping (Jaleel et al., 2021a, b; Lei et al., 2021).

Bio-chemical compounds

Azadirachtin, piperine, and quercetin (95%), utilized in this study, were bought from Sigma Aldrich in St. Louis, Missouri.

Feeding bioassay

Different treatment doses of 10, 5, 2.5, 1.25, and 0.63 µg/ml of azadirachtin, piperine, and quercetin were prepared in20% w/w sugar water. A 20% w/w sugar water was used as the control solution. After 48 h starvation, the *S. invicta* workers received either a single concentration of each plant derivates with the control solution e.g., sugar water. The 1ml solution from each concentration was supplied to these workers continuously via micropipette (1ml) endorsed with in cotton plug until the trial finished in plastic tray. To assess the % mortality data, the dead workers were counted after 24, 48, or 72 h treatment. Each dose had four replicates in a colony. In each replicate, a total of 40 major workers were tested from a colony; this selection was intended to minimize the impact of *S. invicta* worker body size (Xiong et al., 2019).

Statistical procedure

Using "Probit" software, the experiment's results were analyzed which was described by Finney in 1971, the average mortality in each experimental unit was determined (Finney, 1971). The percentage mortality was calculated in MS Excel 2019.

RESULTS

In all treatments, mortality of exposed workers was in direct proportion to concentrations of treatments as well as duration of exposure. We will illustrate only percentage mortality at peak concentrations of each data, while LC_{50} values for each treatment at each interval of recording i.e. 24, 48 and 72 hrs will be presented.

Mortality (%)

In case of azadirachtin, highest concentration (10 ppm) caused maximum mortality i.e., 55.00 %, 62.50 % and 72.50 % of exposed workers after 24, 48 and 72 hrs respectively (Figure 1). Highest concentration (10 ppm) of piperine resulted extreme mortality of fire ant workers i.e. 65.00 %, 77.50 % and 90.00 % after 24, 48 and 72 hrs respectively (Figure 2). Quercetin caused 65.00 %, 70.00 % and 77.50 % mortality of tested workers after 24, 48 and 72 hrs at highest concentration (10 ppm) (Figure 3).

In case of combined treatments, highest concentration (10 ppm) of azadirachtin plus piperine ascribed the highest mortality i.e. 77.00 %, 85.00 % and 92.00 % of workers of fire ants after 24, 48 and 72 hrs respectively (Figure 4). Azadirachtin plus quercetin caused peak mortality i.e. 70.00 %, 80.00 % and 90.00 % of workers at highest concentration (10 ppm) after 24, 48 and 72 hrs of feeding respectively (Figure 5). Highest concentration (10 ppm) of piperine plus quercetin resulted extreme mortality i.e. 70.00 %, 87.50 % and 95.00 % of fire ant workers after 24, 48 and 72 hrs respectively (Figure 6). Highest concentration (10 ppm) of azadirachtin plus piperine plus quercetin was found to cause 65.00 %, 70.00 % and 77.00 % mortality after 24, 48 and 72 hrs respectively (Figure 7).

Toxicity results

In case of azadirachtin, LC_{50} values were recorded as 6.246, 4.244 and 2.033 µg/ml after 24, 48, and 72 hrs of feeding respectively (Table 1). Piperine caused toxicity in exposed *S. invicta* workers with LC_{50} values of 1.984, 0.909 and 0.455 µg/ml after 24, 48 and 72 hrs. LC_{50} values of quercetin were observed as 3.737, 2.327 and 1.616 µg/ml after 24, 48 and 72 hrs respectively.

In case of combined botanicals, azadirachtin plus piperine appeared with LC_{50} values of 1.448, 0.741 and 0.449 µg/ml after 24, 48, and 72 hrs of feeding respectively. While azadirachtin plus quercetin were found LC_{50} values of 2.415, 1.409 and 0.806 µg/ml after 24, 48 and 72 hrs of feeding respectively. Quercetin plus piperine characterized the toxicity of test population with LC_{50} values of 1.594, 0.426 and 0.388 µg/ml after 24, 48 and 72 hrs respectively. In case, when all the botanicals were combined i.e. azadirachtin plus quercetin plus piperine, LC_{50} values were recorded as 3.737, 2.327 and 1.616 µg/ml after 24, 48 and 72 hrs of feeding of test populations.

Over all, highest LC_{50} value 2.033 (95% Cl; µg/ml) after 24 hrs was recorded when fire ants workers fed on azadirachtin mixed sugar solution, which shows less toxic. On the other hand, the lowest LC_{50} value was recorded after 72 hrs i.e., 0.449 when fire ants workers fed on azadirachtin plus piperine, that represents the high toxicity value.

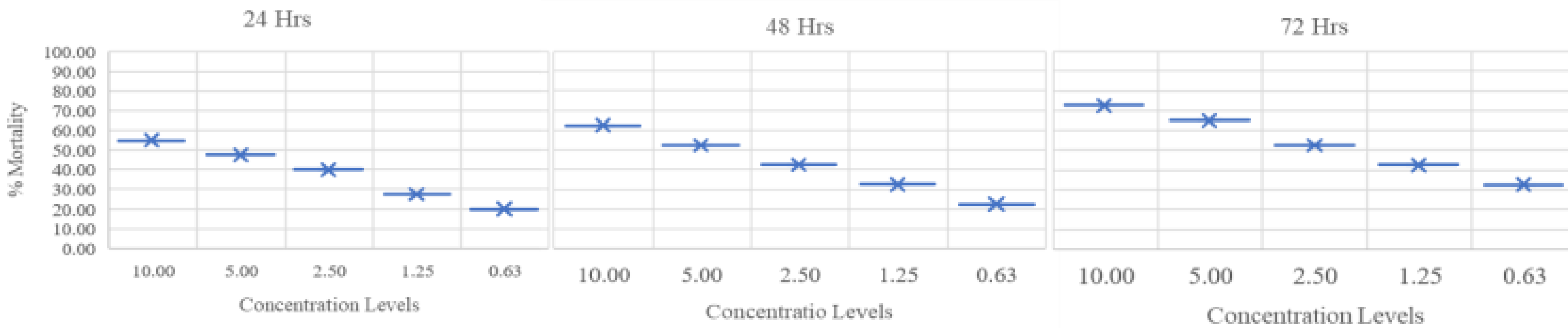

Figure 1. Mortality (%) of *S. invicta* workers after 24, 48, and 72 hrs when treated with azadirachtin.

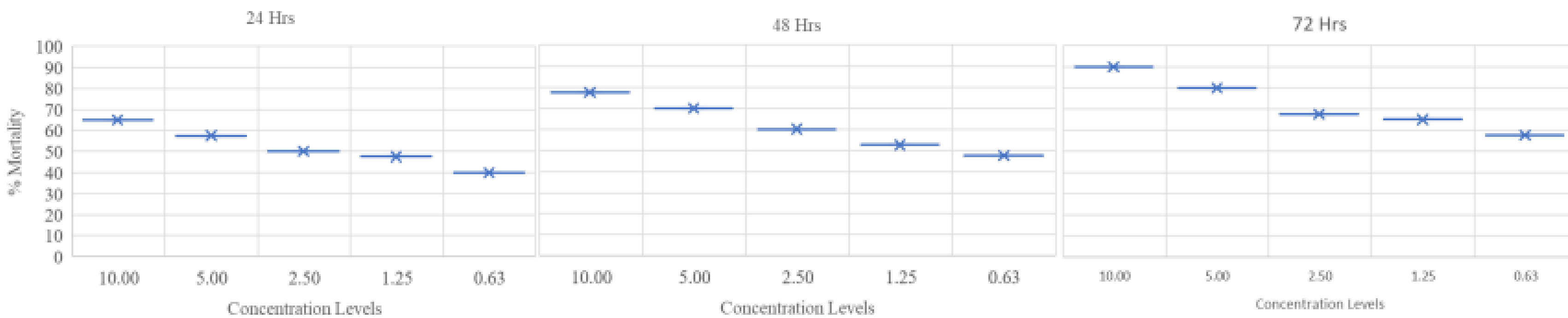

Figure 2. Mortality (%) of *S. invicta* workers after 24, 48, and 72 hrs when treated with piperine.

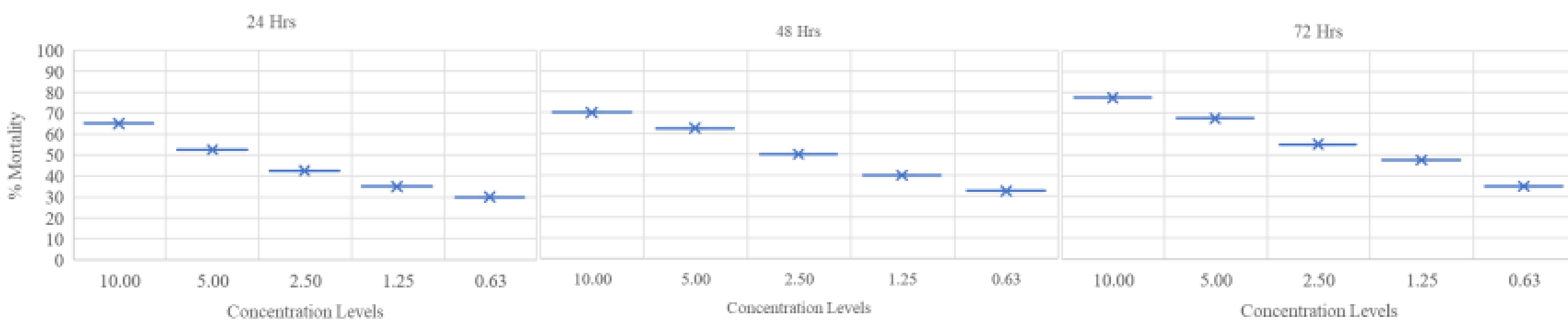

Figure 3. Mortality (%) of *S. invicta* workers after 24, 48, and 72 hrs when treated with quercetin.

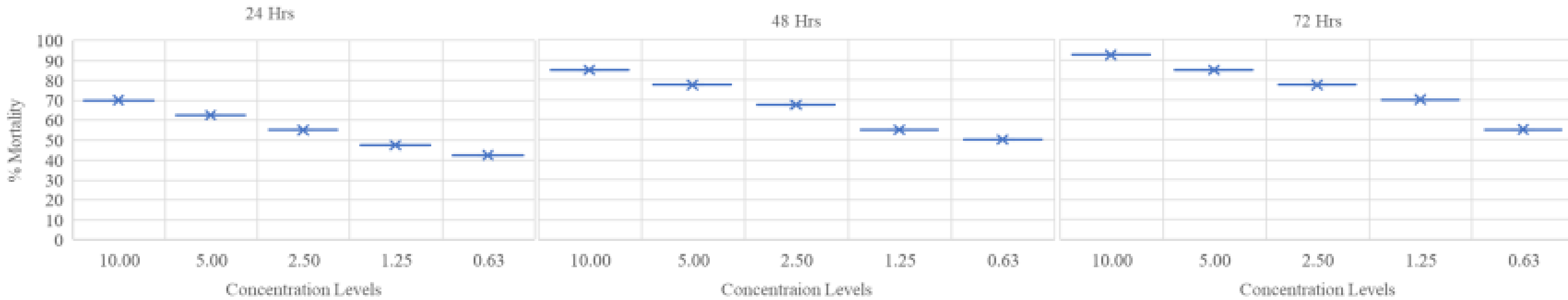

Figure 4. Mortality (%) of *S. invicta* workers after 24, 48, and 72 hrs when treated with combination of azadirachtin + piperine.

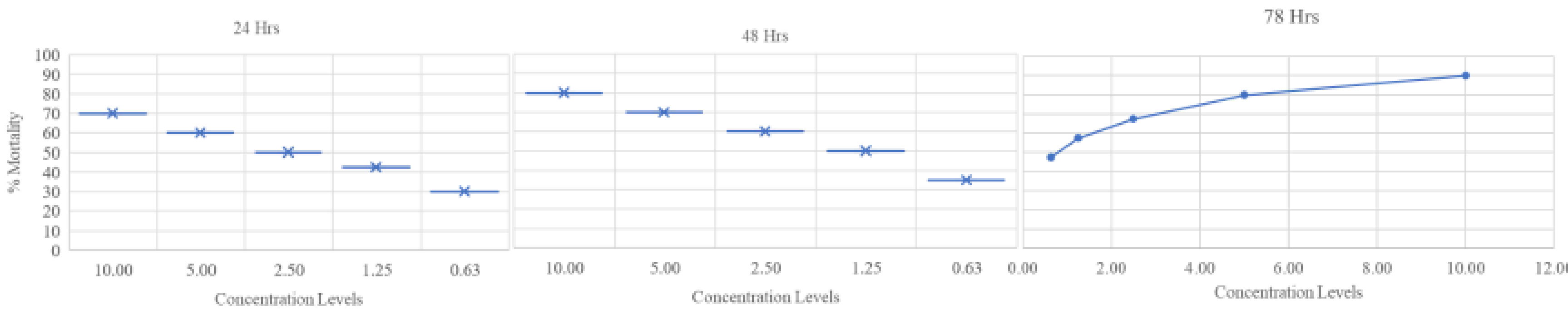

Figure 5. Mortality (%) of *S. invicta* workers after 24, 48, and 72 hrs, when treated with combination of an active ingredient, azadirachtin and a phenolic compound quercetin.

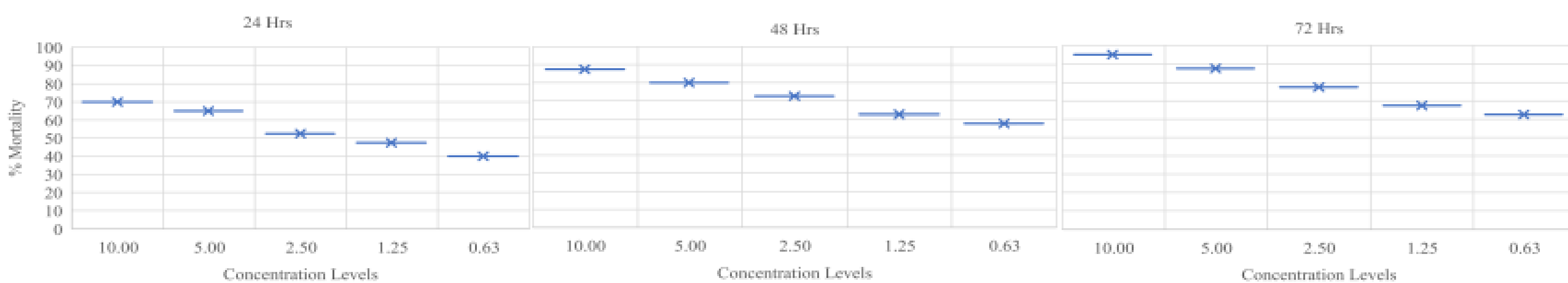

Figure 6. Mortality (%) of *S. invicta* workers after 24, 48, and 72 hrs, when treated with combination of an active ingredient, piperine and a phenolic compound quercetin.

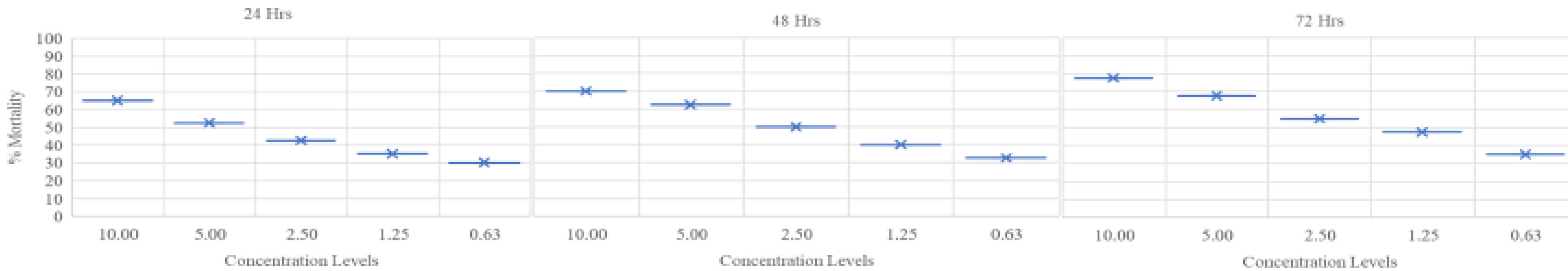

Figure 7. Mortality (%) of *S. invicta* workers after 24, 48, and 72 hrs, when treated with combinations of two active ingredients (azadirachtin and piperine) and a phenolic compound (quercetin).

Table 1. Feeding toxicity of four different bioactive compound and pesticide on RIFA worker.

Bioactive compound	Hrs	LC_{50} (µg/ml)	Slope ± SE	χ^2	*df*
A	24	6.246	0.817±0.025	0.024	3
	48	4.244	0.885±0.220	0,027	3
	72	2.033	0.893±0.218	0.049	3
P	24	1.984	0.508±0.212	0.130	3
	48	0.909	0.694±0.218	0.177	3
	72	0.455	0.838±0.236	1.149	3
Q	24	3.737	0.757±0.216	0.302	3
	48	2.327	0.843±0.217	0.087	3
	72	1.616	0.929±0.220	0.116	3
A + P	24	1.448	0.601±0.214	0.052	3
	48	0.741	0.893±0.228	0.277	3
	72	0.449	1.038±0.250	0.178	3
A + Q	24	2.415	0.844±0.217	0.101	3
	48	1.409	0.991±0.223	0.115	3
	72	0.806	1.086±0.237	0.359	3
P + Q	24	1.594	0.667±0.215	0.196	3
	48	0.426	0.802±0.233	0.201	3
	72	0.388	1.050±0.259	0.804	3
A + P + Q	24	3.737	0.757±0.216	0.302	3
	48	2.327	0.843±0.217	0.087	3
	72	1.616	0.929±0.220	0.116	3

Cl: Confidence limit, χ^2: Pearson's chi-squared goodness-of fit test. df: Degree of freedom A: Azadirachtin, P: Piperine, and Q: Quercetin.

DISCUSSION

Exploration of effective management techniques against *S. invicta* has gained the great concern of researchers because of its well-known negative impacts on public health, economic development and native biodiversity. Among various control measures, use of botanicals is being highly encouraged by scientists because of no threats for non-target organisms and environment (Isman, 2006). In most cases, botanicals are used as deterrents, while in few cases these can be used as stomach toxins. In our current research, we tested the effectiveness of two botanicals and one phenolic compound separately as well as their all possible combinations including azadirachtin, piperine, a phenolic compound "quercetin", azadirachtin + piperine, azadirachtin + quercetin, piperine + quercetin and azadirachtin + quercetin + piperine against *S. invicta* workers.

All treatments were promising against test populations. In case of individual botanical treatments (without combinations), piperine appeared most toxic against test population during each observation. Previous studies also favor our findings. Piperine is major component of black pepper. Bio-pesticidal properties of black pepper is due to piperine and its synergistic relation to other compounds found in black pepper. So, we also used the results of researches revealing the bio-pesticidal activities of black pepper in support of our results. In an experiment, powder of black pepper and its ethanol crude extract resulted in significant mortality of rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) (Su, 1977). Our results are also supported by those of Samuel et al. (2016) who reported the powder of black pepper and piperine as most effective control measure against *Anopheles arabiensis* (Diptera: Culicidae), *A. coluzzii* (Diptera: Culicidae), *A. gambiae* (Diptera: Culicidae), *A. quadriannulatus* (Diptera: Culicidae) and *A. funestus* (Diptera: Culicidae). They found that ground black pepper was effective against resistance strains of *Anopheles* spp. Essential oil obtained from black pepper was used in the mosquito coil against *A. gambiae*. Vaporization of coil caused 76.70 % mortality of exposed test population (Kemabonta et al., 2018). In another laboratory experiment, acetone extract of black pepper was proved an effective repellent against *Bactrocera correcta* (Diptera: Tephritidae) and *B. dorsalis* (Diptera: Tephritidae) (Jaleel et al., 2020).

Quercetin proved 2nd most toxic individual (without combinations of other treatments) botanical against workers of *S. invicta*. It is naturally occurring flavonoid in terrestrial plants and pollens of flowers. It functions as natural deterrent against invader insects but is quite harmless for pollinator insects (Riddick, 2021). Very limited published data is available about the toxicity of quercetin against insect pests. However, work of Jaleel et al. (2020) supports our results who reported the four botanicals including quercetin as effective repellent against two species of fruit flies i.e., *B. correcta* and *B. dorsalis*.

Azadirachtin appeared the 3rd most individual (without combination) toxic botanical against test population. Azadirachtin is obtained from extracts of neem, *Azadirachta indica* (Sapindales: Meliaceae) (Chandramohan et al., 2016; Gupta et al., 2017; Adhikari et al., 2020). So, it will not be unjust to use the results of researches presenting neem as powerful bio-insecticidal plant in favor of effectiveness of azadirachtin. Previous researches also reported azadirachtin as repellent and toxic against large spectrum of insect pests. In an experiment, azadirachtin effectively protected maize crop against western corn root worm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) (Toepfer et al., 2021). Azadirachtin also induces serious behavioral changes in *Drosophila melanogaster* and can be manipulated in management strategies against this insect pest (Bezzar-Bendjazia et al., 2017). In an experiment, neem extract (source of azadirachtin) was proved to be toxic for 3rd instar larvae of stored grain insect pest *Trogoderma granarium* (Coleoptera: Dermestidae) (Satti et al., 2013). In another experiment, neem seed oil extract and methanolic neem leaf extract proved potential bioinsecticides against larvae of fall armyworm (Tulashie et al., 2021). In a field experiment, application of neem extracts protected the cowpea field from various insect pests including whiteflies, aphids and pod borers promisingly (Okolo and Oyewole, 2019).

In case of combined botanicals, combination of piperine with other botanicals (azadirachtin and quercetin) appeared most toxic against test populations. The possible reason is that, as piperine caused highest toxicity when used without combinations, so when it was used in combinations with azadirachtin and quercetin, the highest toxicity was possibly ascribed by piperine. When all of the three botanicals were used in combination, they also caused satisfied mortality of test populations.

CONCLUSIONS AND RECOMMENDATIONS

Our study concluded that piperine is useful bioactive component against red imported fire ant workers so we suggest it should be test in social places as bioactive insecticides against red imported fire ant and should be given proper place in IPM strategies against this pest. However, more work is required to test and improve the effectiveness of such bioactive compounds under field conditions.

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AUTHORS' CONTRIBUTIONS

AR, WJ, MI, MZM, RA, and WM conceived and designed the study; SS conducted surveys and collected the data; AR and WJ analyzed the data; AR, MI, and WJ wrote the manuscript; LA, MAA, DW, SAHR, YH supervised the work and proofread the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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