

Original Research Article

Evaluation of the Efficacy of Chitosan, Certain Essential Oil Extracts, and the Fungus *Penicillium commune* in Controlling the Whitefly, *Bemisia tabaci*, on Cucumber under Laboratory Conditions

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Abstract: **Background:** *Bemisia tabaci* is whitefly with the highest level of economic significance worldwide, has a moderate to disastrous economic. **Aim:** Evaluate safe alternatives to chemical pesticides for controlling the whitefly, *Bemisia tabaci*, infesting cucumber crops. **Methodology:** Several pairs of adult insects (males and females) were collected from cucumber crops grown in greenhouses. Natural chitosan extracts and essential oil extracts from cloves and seeds were used. **Results:** Chitosan demonstrated significant efficacy in reducing susceptibility to infestation compared to the control treatment. The most effective result was observed at a 15% concentration, where the infestation rate decreased to 20.0% for the nymphal stage (compared to 61.0% in the control) and 19.6% for the adult stage (compared to 66.4% in the control). Clove oil treatments recorded high mortality rates, which increased with both concentration and exposure time. The highest mortality rate for nymphs was 86.9% at a concentration of 40 µl/mL, while the highest mortality for adults was 78.8% at the same concentration. Rapeseed essential oil also exhibited significant efficacy, correlating with increased concentrations; the maximum mortality rates recorded were 80.2% for nymphs and 73.1% for adults at a concentration of 40 µl/mL. Treatment with the *Penicillium commune* fungal filtrate induced high mortality rates that increased over the exposure period (1, 3, and 5 days). **Conclusion:** Biocontrol agents and botanical extracts (chitosan, essential oils, fungal filtrate) demonstrated high efficiency in controlling the whitefly, *B. tabaci*. This makes them safe and effective alternatives to chemical pesticides that negatively impact the environment and public health.

Keywords: Chitosan, Whitefly, Cucumber, Clove Oil, Essential Oils, *Penicillium Commune*.

INTRODUCTION

Bemisia tabaci is one of the 1,556 species of the whitefly with the highest level of economic significance worldwide (Perring *et al.*, 2018; Alessandro *et al.*, 2016). The whitefly is regarded as a polyphagous insect that feeds on different crops of the family Solanaceae, including pepper, tomato, potato, and tobacco (Kunjwal *et al.*, 2018; Khan *et al.*, 2015). *B. tabaci* has a moderate to disastrous economic impact that results in annual losses of billions of dollars on numerous crops (Mascarin *et al.*, 2013; Hasan *et al.*, 2019).

Cucumber, *Cucumis sativus* L., is considered one of the most significant and widely cultivated summer vegetable crops globally, particularly in Iraq. Belonging to the family Cucurbitaceae, its origins are believed to trace back to Africa and India (Al-Sahaf, 2011). In Iraq, cucumber is cultivated in open fields during two seasonal cycles (spring and autumn), as well as in protected environments including low tunnels, polyethylene houses (polyhouses), and greenhouses. According to data provided by Central Statistical Organization (2014), in 2014, the total area covered by cucumber increased to 82,218 dunams with an overall production of 56,334 tons. Owing to the economic value of the crop and increasing consumption, plant breeders have shown a lot of interest in this crop to come up with hundreds of hybrids that have high yield, good quality, resistant to pests, etc. Introduction of hybrids is considered as one of the key measures of breeding and betterment of plants (Hassan, 2005).

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Cucumber plants are vulnerable to attack by many pests that cost the economy through losses, among them the red pumpkin beetle, cucumber beetle, strawberry mite and the whitefly (Al-Azzawi, 1990), as well as thrips including Thrips palmi and Thrips tabaci (Al-Jurani, 2013). The first factors that determine the severity of the pests are environmental conditions; what may be a secondary pest in an open field may become a major pest in the same crop in different climatic conditions. This has been made possible after the increase of vegetable cultivation in green houses and plastic houses where tomato leaf miners have become economical pests on the host plants under the conditions of protection, especially to cucumber and tomato plants (Abul *et al.*, 1961; Man *et al.*, 2007).

The application of synthetic chemical pesticides has been common since the 1950s because of its ability to curb the insect pests. Nevertheless, they have had severe negative impacts when used too much and too widely such as poisoning, pollution of the environment and damaging other organisms that are not targets like bee and fish (Paoletti and Pimentel, 2000). Despite the prevalence of chemical control as the most widely used approach to address the *B. tabaci*, there are growing concerns about the threats of chemicals to the health of the consumers and the emergence of resistance in the pest, which is why more and more researchers tend to turn to the use of biological control agents such as fungi and plant extracts as safer options (Skinner *et al.*, 2013). Thus, the purpose of the present study was to use the chitosan, essential oil extracts of clove and rapeseed seeds as well as the fungus *Penicillium commune* to manage whitefly.

MATERIALS AND METHODS

Insect Collection and Rearing

Several pairs of adult insects (males and females) were collected from cucumber crops cultivated in plastic houses (polyhouses). They were transferred to a rearing cage measuring 200 × 60 × 120 cm, covered with muslin cloth on the sides. Potted cucumber plants were placed inside the cage to serve as a food source for the insects.

Preparation of Chitosan Concentrations

Natural chitosan extracted from shrimp shells was utilized in this experiment. The product (a fine white powder) was obtained from a scientific bureau in a 900 g package produced by ZOOS Victoria. To prepare the solution, 20 g of chitosan was dissolved in 50 mL of acetic acid, and the volume was made up to 1000 mL with distilled water to prepare the concentrations used in the study. The solution was applied as a foliar spray on the vegetative parts of the plants until runoff (complete wetness) in the early morning using a 20-liter manual sprayer. Two applications were administered, with the second application occurring ten days after the first. A few drops of liquid soap were added as a surfactant. The percentage of infestation was calculated using the following formula:

$$\text{Infestation Percentage (\%)} = \frac{\text{Number of Infested Plants}}{\text{Total Number of Plants}} \times 100$$

Extraction of Essential Oils from Clove (*Syzygium aromaticum*) and Rapeseed (*Brassica*)

The essential oils were extracted from the seeds of clove and rapeseed using a modified steam distillation apparatus (Clevenger-type, Fine herm, China). One hundred grams of the plant material was ground using an electric grinder at 2500 rpm shortly before extraction or field application to prevent the loss of volatile active compounds. One hundred grams of the powder was immersed in 700 mL of distilled water in a 1-liter glass flask within the apparatus. The temperature was initially set to the boiling point of water (100 °C). After 20 minutes, the temperature was reduced to 65 °C to facilitate extraction via evaporation and condensation while preserving the quality of the active compounds. The extraction process lasted three hours for each sample. Careful attention was paid to adjusting the temperature and exposure time to suit the type of oil being extracted. The water in the flask served to prevent overheating. The steam carrying the essential oil rose, condensed in the cooling coil, and collected in the receiver. The vital oil, which was less dense than water, floated on it. It was gathered by draining the accumulated water using the stopcock until the level of oil was towards the valve. The oil was subsequently kept in dark glass airtight containers at 3 °C (Tongnuanchan and Benjakul, 2014; Ghafoor and Faraj, 2017).

Clove and rapeseed gave yield of 5 mL and 6 mL respectively of the plant powder per 100 g of the sample. The extraction was carried out a number of times to get adequate amounts. Any water that was left was eliminated with the use of a 1 mL syringe (insulin syringe) and anhydrous sodium sulfate was incorporated into the essential oil in order to eliminate any form of suspended water droplets and leave the extract 100% pure and usable. To make the stock solution (80 mg/mL), 8 mL of the individual extracts was put independently and mixed with 5 mL of chloroform and stirred. Surfactant (Tween 20) 3 mL were added, and batches from 100 mL of distilled water were added. Based on this stock solution, the levels of 10, 20, and 40 µg/mL of the solution were made, with the treatment of the control being that of the pure distilled water.

Culture Media

Potato Dextrose Agar (PDA)

A water bath was used to dissolve 39g of ready-made PDA (Himedia, India) in 1 liter of distilled water in a 1-liter glass flask. Tetracycline, an antibiotic was put in a concentration of 250mg/L. The medium was made in 250 mL glass

flasks which were plugged with cotton wool and sterilized in a 121 °C and 15 psi pressure and 20 minutes in an autoclave. The media was put into a refrigerator after cooling, until it could be used to isolate, purify and propagate the fungi.

Preparation of Potato Dextrose Broth (PDB)

Tubers of the potato were washed, peeled and cut into small pieces. Two hundred grams of the prepared potatoes were boiled in 500 mL of distilled water in a 1-liter glass beaker over a period of 20 minutes. The mixture was filtered using muslin cloth. Dextrose (20 grams) was put in the filtrate and the final volume was put to 1 liter with distilled water. The broth was separately transferred into 250 mL glass flasks at 150 mL per flask. The flasks were autoclaved at 121 °C and 15 psi pressure, where it was kept at 20 minutes and later used to prepare fungal filtrate.

Preparation of *Penicillium commune* Fungal Filtrate

After preparing the liquid growth medium (as mentioned in section 2) and pouring it into flasks (150 mL/flask), the antibiotic Tetracycline was introduced at the concentration of 250 mg/L. Three mycelial discs (cut with a cork borer) were inoculated into each and every flask with a fungal culture that was 7 days old. The flasks were left at 25±2 °C and swirled after every 3-4 days to facilitate the equal growth of fungi. The culture was filtered after 28 days with Buchner funnel and vacuum pump through the Whatman No.1 filter paper. This was again sterilized in a Millipore microfilter. The filtrate that was obtained was put in tubes and refrigerated to be used in later experiments. The three concentrations of the filtrate (25%-50 and 75) were made by withdrawing the volume of the stock filtrate using sterile medical syringe and adding 100 mL of sterile distilled water.

Statistical Analysis

The data collected in the experiment were examined as a factorial experiment based on a Completely Randomized Design (C.R.D.). The significance of difference in means was estimated using the Least Significant Difference (L.S.D.) test at the probability level of P 0.05. To eliminate the biases on the mortality percentages, the formula of Abbott was used (Abbott, 1925).

RESULTS AND DISCUSSION

Findings presented in (Table 1) suggest that there were large variations between the chitosan treatment and the control treatment when it comes to the impact on infestation of plant life, in respect to the level of concentration used, namely the 15% concentration whereby the infestation rate was 20.0%, as against a pre-treatment rate of 61.0%. The least efficacy on the other hand was at the 5% concentration with an infestation rate of 48.1% as compared to the control. Concerning the adult stage, the outcomes also showed great differences between the chitosan treatments and the control. The rate of infestation dropped to 19.6% at the 15% concentration (as compared to the 66.4% infestation rate at pre-treatment stage) but the 5% concentration produced an infestation rate of 41.0% as compared to control.

Table 1: Effect of Chitosan on the Susceptibility of Different Stages of the Whitefly, *Bemisia tabaci*, to Infestation

Stage	Concentration (%)	Initial Infestation Rate	Infestation Rate After Treatment
Nymphal stage	0	00.0	00.0
	5	61.0	48.1
	10	55.2	31.3
	15	41.5	20.0
L.S.D: 0.05		2.5	4.3
Stage	Concentration (%)	Initial Infestation Rate	Infestation Rate After Treatment
Adult stage	0	00.0	00.0
	5	67.5	41.0
	10	59.0	28.9
	15	66.4	19.6
L.S.D: 0.05		4.1	5.2

The same effect was confirmed by Ikram *et al.*, (2017), which reached the conclusion that it is the resistance-inducing properties of chitosan. It has been applied to manage diseases and pests, reduce their spread and enhance defense of the plants by activating host responses. Such reactions are improved formation of callus, prevention of proteinase enzymes, lignification, stimulation of chitinase, synthesis of phytoalexin, and expression of defenses genes besides its antioxidant activity. Moreover, this compound has attracted a lot of attention owing to the high level of nitrogen (about 6.89%), which makes it an advantageous chelating agent to many nutrients. Chitosan can easily be transformed into emulsions, films, hydrogel and edible covers. The initial research describing chitosan as fungicide was done in 1979 by Allan and Hadwiger who indicated that chitosan was able to block the growth of the fungal cell wall and hence interest was raised by the industries in agricultural sector.

Chitin and chitosan are biodegradable, antimicrobial, antioxidant and have found numerous applications in different industries. Other studies have reported that chitosan is better than chitin in the inhibition of fungal growth (Tanha *et al.*, 2017). It has been used in food industry, as a preservative and also coating agent to protect the fruits against contamination by its antimicrobial property (Uthairatanakij *et al.*, 2007; Ali and Mehmood, 2019). In addition, the US Food and Drug Administration (FDA) has categorized chitosan as safe in the use of wound dressing (Aljebory and Alsaman, 2017).

Kadhim (2020) showed that when chitosan was applied to Vinca plants at the concentration of 40% on the foliage, most of the traits studied increased significantly. It is worth noting that the need to use environmentally friendly compounds in improving agricultural development and productivity is increasingly important in ensuring a transition towards clean agriculture and neglect of the use of harmful chemicals. On the same note, Al-Saadi (2021) pointed out the possible uses of chitosan polymers in other sectors, such as the medical sector where they can be used as vehicles that deliver drugs. Al-Rammahi (2021) also suggested to use conventional and nano-urea, conventional chitosan at the physiological maturity stage. This is recommended using their capability in ensuring the grains of maize are not infested by fungi and pests, they are dried by lowering the moisture level in the field, and they enhance the storage life. More so, they can be used as aerial sprays on extensive regions.

(Table 2) demonstrates the impact of the clove essential oil on the mortality of both adult and nymphal stages. It was noted that as the concentration of the extract was changed the mortality rate of both stages similarly increased. The maximum and minimum mortality rates of the nymphal stage were 86.9% and 42.4% respectively at 40 and 10 $\mu\text{l/mL}$ concentration respectively. On exposure time, there was an increase in death rate with the exposure duration, which reached 44.4%, 48.7% and 53.7% following 1, 5 and 7 days of exposure respectively on nymphal stage. On the adult phase, 78.8 at the 40 $\mu\text{l/mL}$ concentration was the highest mortality rate and 22.7 at 10 $\mu\text{l/mL}$ was the lowest mortality rate. In the same way, time factor effect led to mortality rates of 35.5, 39.9 and 46.2 in the 1st, 5th and 7th days respectively. On the interaction of the insect stages, clove seed essential oil concentration, and the exposure periods, the findings showed that an increase in the extract concentration and exposure time showed an increase in the mortality rates. The highest percentage recorded was 86.9% with nymphs at 40 $\mu\text{l/mL}$ with a minimum of 42.4% at 10 $\mu\text{l/mL}$. In adults, the highest mortality was 78.8% at 40 $\mu\text{l/mL}$ and at the lowest, 22.7% at 10 $\mu\text{l/mL}$.

Table 2: Effect of Clove (*Syzygium aromaticum*) Seed Essential Oils on the Mortality Rate of Nymphal and Adult Stages of the Whitefly, *Bemisia tabaci*, under Laboratory Conditions

Treatment	Concentrations ($\mu\text{l/mL}$)	Mortality Percentage (%) / Day			Mean of Concentrations
		Concentrations ($\mu\text{l/mL}$)			
Nymphal stage		1	5	7	
	Control	0.0	0.0	0.0	0.0
	10	42.4	50.4	58.0	50.3
	20	62.4	68.8	70.0	67.1
	40	72.9	75.5	86.9	78.4
Mean of Time		72.9	75.5	86.9	
L.S.D: 0.05 Concentrations: 5.2 For periods: 3.1 For interference: 4.1					
Adult stage	Control	0.0	0.0	0.0	0.0
	10	34.9	34.9	44.1	33.9
	20	54.8	54.8	62.2	55.6
	40	70.1	70.1	78.8	72.8
Mean of Time		35.5	39.9	46.2	
L.S.D: 0.05 Concentrations:4.4 For periods: 3.4 For interference:3.6					

These findings are in line with Goswami and Khan (2013) concerning the acaricidal effects of essential oils especially clove oil. The same level of consistency is also applied to the *in vitro* study of Gashout and Guzman-Nova (2009), who showed that Varroa mites had a 96% mortality on clove oil (in combination with menthol and oregano). Also, our results are in agreement with Hoppe (1990) who indicated that fumigation with clove oil led to 100% death of Varroa parasites following 24 hours of treatment. Additionally, the information is consistent with that of Ruffinengo *et al.*, (2001) that proved that the toxicity of essential oils increases by a significant number when exposed to the same compounds over a long period of time. These results, however, do not correlate with Dawood *et al.*, (1991), who reported that eucalyptus leaf oil was the top most extract among those tested which obtained a 73% mortality rate in the adult southern cowpea beetles.

(Table 3) shows the impact on the death of the nymphal and adult stages of rapeseed (*Brassica napus*) seed essential oils. It was noted that the higher the concentration of the extract, the more the mortality rate was increased in the two stages. For the nymphal stage, the highest mortality rate was 80.2% at a concentration of 40 $\mu\text{l/mL}$, while the lowest

rate was 30.1% at 10 $\mu\text{l/mL}$. Regarding the effect of exposure time on nymphs, mortality rates increased with duration, recording 36.5%, 40.8%, and 45.9% after 1, 5, and 7 days, respectively. As for the adult stage, the highest mortality rate (73.1%) was recorded at a concentration of 40 $\mu\text{l/mL}$, whereas the lowest rate (20.1%) was observed at 10 $\mu\text{l/mL}$. Similarly, the mortality rates for adults increased with exposure time, reaching 31.1%, 35.6%, and 39.2% after 1, 5, and 7 days, respectively. Finally, regarding the interaction between the growth stage, oil concentration, and exposure time, the results indicated that increasing both the extract concentration and the exposure duration led to a significant increase in mortality rates."

Table 3: Effect of essential oils of rapeseed (*Brassica napus*) on the percentage of nymphal and adult mortality of the whitefly *B. tabaci* in the laboratory

Treatment	Concentrations ($\mu\text{l/mL}$)	Mortality Percentage (%) / Day			Mean of Concentrations
		Concentrations ($\mu\text{l/mL}$)			
Nymphal stage		1	5	7	
	Control	0.0	0.0	0.0	0.0
	10	30.1	36.2	44.0	36.8
	20	50.8	55.9	59.5	55.4
	40	64.9	71.1	80.2	72.1
Mean of Time		36.5	40.8	45.9	
L.S.D: 0.05 Concentrations:5.0 For periods:4.5 For interference:3.6					
Adult stage	Control	0.0	0.0	0.0	0.0
	10	20.1	29.9	33.3	27.8
	20	44.2	46.1	50.5	46.9
	40	59.9	66.4	73.1	66.5
Mean of Time		31.1	35.6	39.2	
L.S.D: 0.05 Concentrations:4.8 For periods: 3.0 For interference:2.5					

These results are consistent with what Ahmed *et al.*, (2010) concluded, that mortality rates increase with increasing concentration of the extract, by studying the toxicity of seven essential oils, namely tea, cinnamon, clove, lemongrass, eucalyptus, thyme, and jojoba, against the two insects, the rice weevil (*Sitophilus oryzae* L.) and the cowpea beetle (*Callosobruchus maculatus* L.). The study concluded that mortality rates increase with increasing concentration and exposure time. Begonia (2009) demonstrated that the use of 1% Omini oil was highly effective in laboratory and field tests, outperforming a mixture of rosemary and peppermint oils (Ecterol) and the acaricide Nexter against the mobile and egg stages of the grape mite (*Eotetranychus willamettei*). All essential oils have a similar mechanism of action: they kill insect pests and mite species by preventing gas exchange between the external environment and the body wall, as well as by preventing feeding due to covering plant tissues. They also prevent embryonic respiration in the egg by coating the egg wall, causing it to harden and preventing gas exchange, thus killing the embryo due to lack of oxygen (Johnson, 1991).

(Table 4) shows the effect of *P. commune* fungal filtrate concentrations and treatment time on the percentage mortality rate of *B. tabaci* whitefly nymphs. Statistical analysis revealed significant differences resulting from the effect of *P. commune* fungal filtrate concentrations on the percentage of nymph mortality. The highest mortality rate was 82.5% at a concentration of 75%, while the lowest was 34.1% at a concentration of 25%. It was also observed that the mortality rate increased with increasing treatment time, reaching 37.0%, 42.3%, and 50.3% after 1, 3, and 5 days, respectively. Regarding the interaction, the highest mortality rate was 90.0% at a concentration of 75% on the fifth day, compared to 0.0% in the control treatment on the fifth day.

Table 4: Effect of different concentrations of *P. commune* fungal filtrate on the percentage mortality rate of the nymphal stage of the whitefly *B. tabaci* in vitro

Concentrations %	Time period (days)			Concentration rate
	1	3	5	
0	0.0	0.0	0.0	0.0
25	26.2	34.9	41.1	34.1
50	45.3	53.4	69.9	56.2
75	76.6	81.0	90.0	82.5
Time period rate	37.0	42.3	50.3	
LSD value (0.05) for concentrations		5.4		
L.S.D. value (0.05) for the time period		4.2		
L.S.D. value (0.05) for interference		3.6		

The high mortality rate of nymphs and adults of the whitefly *B. tabaci* may be attributed to the active compounds in the filtrate of the fungus *P. commune*, such as cyclopeptin, cyclophenin, cyclophenol, viridicatol, viridicatin, and cyclopaldic acid, which are similar to those found in manufactured pesticides but without environmental impact. It may also be due to the fungus's ability to secrete enzymes that break down the body wall, such as chitinase, protease, and lipase, which play a crucial role in the insect's destruction. The mortality rate may also be attributed to the fungus's secondary metabolites, which can interfere with the immune system, leading to changes in host behavior such as reduced feeding and activity, paralysis of the insect, and alterations in tissue structure, ultimately resulting in the host's rapid death (Charnley, 2003). (Table 5) shows the interaction effect between *P. commune* fungal filtrate concentrations and treatment time on the percentage mortality rate of adult *B. tabaci* whiteflies. Statistical analysis revealed significant differences in adult mortality resulting from *P. commune* fungal filtrate concentrations. The highest mortality rate was 69.9% at a 75% concentration, while the lowest was 26.1% at a 25% concentration. It was also observed that mortality increased with each additional treatment time, reaching 30.5%, 35.5%, and 42.1% after 1, 3, and 5 days, respectively. Regarding the interaction, the highest mortality rate was 77.8% at a 75% concentration on day 5, compared to the control treatment.

Table 5: Effect of Different Concentrations of *P. commune* Fungal Filtrate on the Mortality Rate of the Adult Stage of the Whitefly, *Bemisia tabaci*, under Laboratory Conditions

Concentrations %	Time period (days)			Concentration rate
	1	3	5	
0	0.0	0.0	0.0	0.0
25	19.9	25.2	19.9	26.1
50	39.0	47.9	39.0	48.0
75	63.1	68.8	63.1	69.9
Time period rate	30.5	35.5	42.1	
LSD value (0.05) for concentrations	4.1			
L.S.D. value (0.05) for the time period	3.5			
L.S.D. value (0.05) for interference	3.8			

(Charnley, 2003). Furthermore, increasing the concentrations of fungal toxins leads to a higher mortality rate following the entry of these toxins through contact with the integument, oral ingestion, or the spiracles. Alternatively, this can occur through the direct penetration of the fungus into the body wall. It has been found that the most susceptible areas for penetration are the intersegmental membranes. This penetration weakens the insect's defense mechanisms, thereby increasing its susceptibility to infection. Furthermore, an increase in the concentrations of mycotoxins secreted by the fungus, such as cyclopiazonic acid, leads to higher mortality rates. This occurs following the entry of these toxins via contact with the integument, oral ingestion, the spiracles, or through direct fungal penetration of the body wall. It appears that the time required for *P. commune* to cause insect mortality depends on the physical nature of the outer cuticle. For individuals possessing a hard (sclerotized) cuticle, the fungus requires a longer period to kill the pest, whereas individuals with a less rigid (softer) cuticle require less time to be eradicated (Hatem, 2020).

This is in agreement with the findings of Al-Shuwayli (2010), who used the fungal filtrate of *B. bassiana* against the black bean aphid, *Aphis fabae*. A 75% filtrate concentration resulted in mortality rates of 54.10% and 52.17% for nymphs and adults, respectively. The mortality is attributed to the fact that increasing the concentration of the fungal filtrate leads to the accumulation of toxic substances within the insect's cells, causing them to rupture, which consequently increases the mortality rate. These results are also consistent with a study conducted by Al-Jubouri (2007), which demonstrated that true fungi (Eumycota) possess the ability to attack insects by secreting cuticle-degrading enzymes, such as lipase, chitinase, and protease. Furthermore, the current study aligns with the findings of Khalaf *et al.*, (2013), where the *Trichoderma harzianum* fungal filtrate caused mortality in both nymphs and adults of the wheat aphid. For nymphs, the mortality rates were 31.49%, 41.44%, and 46.30% at concentrations of 25%, 50%, and 75%, respectively. For adults, the mortality rates reached 28.05%, 37.00%, and 42.30% at the same respective concentrations (25%, 50%, and 75%).

CONCLUSION

Biocontrol agents and botanical extracts (chitosan, essential oils, and fungal filtrate) demonstrated high efficiency in controlling the whitefly, *B. tabaci*. This makes them safe and effective alternatives to chemical pesticides that negatively impact the environment and public health. The chitosan compound showed a clear effectiveness in increasing the insect's susceptibility to infection and reducing its population. Essential oils (clove and rapeseed/canola) possess strong insecticidal properties against the whitefly, as the mortality rate was directly proportional to the increase in concentration and exposure time. Clove seed essential oil outperformed rapeseed oil in mortality rates. The fungal filtrate demonstrated highly destructive efficiency that surpassed the other treatments, especially at the 75% concentration on the fifth day of exposure, achieving a mortality rate of 90.0% for nymphs and 77.8% for adults. The lethal effect of the fungal filtrate is attributed to

its active compounds and mycotoxins, in addition to the secretion of cuticle-degrading enzymes (such as chitinase, protease, and lipase). This leads to the penetration of the integument and the destruction of the insect's tissues.

RECOMMENDATIONS

It is necessary to conduct field studies (in open fields and greenhouses) to evaluate the efficiency of chitosan (at 15% concentration), clove oil (at 40 µl/mL concentration), and fungal filtrate (at 75% concentration) under natural environmental conditions and compare them with the laboratory results. Incorporate these biological and botanical compounds into Integrated Pest Management (IPM) programs to control the whitefly, *B. tabaci*, on vegetable crops, aiming to reduce reliance on synthetic chemical pesticides. Conduct future research to study the interaction (mixing) effect between chitosan and essential oils, or between chitosan and the fungal filtrate, to determine if mixing will provide a synergistic effect that increases mortality rates at lower concentrations. Study the side effects of these extracts and fungal filtrates on the natural enemies (predators and parasitoids) associated with the whitefly in the field to ensure the specificity and environmental safety of these alternatives. Direct attention towards isolating and purifying the active compounds and mortality-causing enzymes from the *P. commune* fungal filtrate, and explore the possibility of formulating them commercially as biopesticides that are easy to apply and store.

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