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Effects of ZnO nanoparticles and Kaolin in combination with NeemAzal-T/S against *Bemisia tabaci* and its parasitoid *Eretmocerus mundus* on cotton

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ABSTRACT

The cotton whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an important pest of cotton which by transmitting plant pathogenic viruses cause damage and reduce lint quality. To reduce the use of chemical insecticides, effects of ZnO nanoparticles, Kaolin alone or in pairwise combination with NeemAzal-T/S against egg and second instar nymph of *B. tabaci* and pupae of its parasitoid *Eretmocerus mundus* Mercet were evaluated. The LC_{50} values of ZnO NPs, Kaolin and NeemAzal-T/S against eggs and nymphs of *B. tabaci* were 7.49 mg L^{-1}, 24.89 g L^{-1}, 6.83 mg L^{-1} AZA and 6.93 mg L^{-1}, 18.36 g L^{-1} and 6.00 mg L^{-1} AZA 3 days after treatment, respectively. The LC_{50} values of ZnO NPs, Kaolin and NeemAzal-T/S against *E. mundus* were 11.30 mg L^{-1}, 41.59 g L^{-1}, 36.90 mg L^{-1} AZA, respectively 3 days after treatment. In the laboratory conditions, ZnO + NeemAzal and Kaolin + NeemAzal exerted a higher level of control on eggs and nymphs of the pest than either alone, while they had a lower level of negative effects on the parasitoid pupae, too. In the field conditions and at 7 DAT, Kaolin + NeemAzal was the most effective treatment on eggs of the pest, causing a 67.43% reduction, while NeemAzal-T/S was the most effective treatment on nymph (86.52% reduction), which was not different with ZnO + NeemAzal and Kaolin + NeemAzal. NeemAzal and ZnO exerted the highest and lowest mortality on pupae of the parasitoid, respectively. Based on the field studies, ZnO NPs at 20 mg L^{-1}, Kaolin at 30 g L^{-1}, NeemAzal-T/S at 15 mg L^{-1} AZA and mixing equal volumes of NeemAzal-T/S (7.5 mg L^{-1} AZA)+ZnO NPs (10 mg L^{-1}) and NeemAzal-T/S (7.5 mg L^{-1} AZA)+ Kaolin (15 g L^{-1}) can be suitable candidates in IPM programs of *B. tabaci* field condition.

1. Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a serious pest of over 600 different plant species of vegetable and ornamental crops including cotton, eggplant, tomato, beans, soybean and broccoli in many countries [1, 2]. Both adults and nymphs of *B. tabaci* causes direct damage through ingestion of phloem sap or indirect damage by encouraging the growth of sooty mould and by transmitting of over 110 plant viruses [3]. Because of development resistance in *B. tabaci* populations to different classes of insecticides, and negative environmental impacts of chemical insecticides, alternative pest management strategies should be developed to control this pest [4, 5]. *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) is a primary ecto-endo parasitoid of whitefly nymphs [6], this parasitoid is usually unable to maintain whitefly populations below economic injury levels, therefore supplemental treatments are often need in field conditions [7, 8]. In recent years, the application of nanotechnology in insect pest management has been underlined [9]. Nanoparticles (NPs) are ultra-fine particles which have at least one dimension less than 100 nm. NPs are more reactive than their bulk counterpart because of their increased surface to volume ratio [10] and a number of physical properties

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of these materials change as their size approach nanoscale. NPs help to produce new pesticides, insecticides and insect repellents [11, 12]. Among the inorganic metal oxide NPs, Zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention for medical, chemical, industrial and agricultural applications due to their exceptional properties, including low cost synthesis, antibacterial and anti-fungal activity [13, 14]. So, due to the antimicrobial, antioxidant and electrical conductivity characteristics of ZnO NPs, it can be used in active and intelligent biodegradable films [15, 16]. In spite of the studies performed on NPs for control of insect pest species that impact public health or agriculture, little research has been carried out to investigate the toxicity effect of ZnO NPs on whiteflies and natural enemies. Furthermore, based on new and significant properties of NPs, these materials are widely used in agricultural sector; therefore, assessment of their potential toxic effects on environment seems quite necessary [17]. Kaolin is a fine-grained, non-abrasive white aluminum silicate natural clay mineral that readily dissolves in water [18]. When plants are sprayed with kaolin, a physical barrier is formed on plant surfaces that can act as a deterrent to insect settling, oviposition and feeding [19]. As a natural and inert product, kaolin has been considered safe for the environment [20], with the advantage of having low or null effects on beneficial arthropods [21, 22]. Besides, kaolin clay can protect the plant from weather conditions like heat stress and sunburn [23, 24]. Nevertheless, some studies note that the combination of kaolin with other products could improve their efficacy as insecticides [25], so it is possible that the combined application of both products within an IPM program could enhance the efficacy of this insecticidal soap in the field. In some cases, compatible products could be combined to produce higher control efficiency while decreasing the amount of conventional insecticides and minimizing the risks of environmental contamination and insecticide resistance [26]. Neem contains azadirachtin, a triterpenoid derived from neem trees (*Azadirachta indica*), acts as a strong antifeedant and repellent, delays and prevents moulting, reduces growth, development and oviposition, and causes high mortality in a diverse group of phytophagous insects, including *B. tabaci* [27, 28]. It is widely used around the world today either as a stand-alone treatment [28, 29] or in combination with synthetic pesticides [30]. Furthermore, use of such materials could provide a cheap, reliable and safe alternative strategy to combat resistant pests.

Thus, present study was evaluated to examine the efficacy of ZnO NPs, kaolin applied either alone or in pairwise combination with NeemAzal-T/S on egg and second instar nymph of *B. tabaci* and pupae of *E. mundus* under laboratory and field conditions.

2. Materials and methods

2.1. Experimental materials

2.1.1. Physical agents

Zinc oxide nanoparticles (99+%, US Research Nanomaterials, Inc., Twig Leaf Lane, Houston, USA) with size ranging from 10 to 30 nm was used in field and laboratory experiments.

Processed Kaolin (Sepidan® WP, active ingredient (a.i.) 95%) was obtained from Kimia Sabz Avar Co., Iran.

2.1.2. Botanical insecticide

A commercial neem product, NeemAzal-T/S® EC 1% (10000 mg a.i. /L containing azadirachtin A (AZA); Trifolio-M GmbH, Lahnau, Germany) was used for experiments.

2.2. Laboratory experiments

2.2.1. Laboratory conditions and planting

All experiment carried out under 24 ± 2 °C, a 16: 8 h (L: D) photoperiod and 60 ± 5 % RH. For the laboratory experiments, cotton seeds were grown in plastic pots (20 cm depth and 20 cm diameter) in a greenhouse at 16-25 °C, 40 -50% RH under a 14:10 (L:D) photoperiod in a growth chamber and used at the 4-5 leaf stage in the experiments.

2.2.2. Insect cultures

The *B. tabaci* and the parasitoid *E. mundus* was both originally collected from cotton plants, free of insecticides, grown in the research field of scientific staff of Cotton Research Center of East Iran, Kashmar in July 2019, then transferred to growth chamber and cultured in a muslin-walled cage (120×60×60 cm) containing 40 potted cotton plants (cultivar *Varamin*). The parasitoid provided weekly with new nymphs of sweet potato whitefly on leaves of cotton. The rearing conditions were identical to those for whiteflies. Adult of whiteflies were collected from the laboratory colony by an aspirator and transferred to the plastic tubes of 10 cm dep and 3 cm diam. The tubes incubated at 10 °C for 5 minutes in order to the adults be handled more easily, then groups of 30 adults were released into a clip cages confined in to the lower surface of the potted cotton leaves for 24 h. Each clip cage made from 2 plastic container of 5.5 cm dep and 8 cm diam; one in upper, another in lower surface of the cotton leaves, fixed with an elastic string. Each container ventilated by means of a fine mesh glued to an aperture cut in the bottom (5 cm diam). Afterward, adults were removed from the cages and leaves have eggs on them were labeled and used in the bioassay or incubated at 24±2 °C, 60±5% RH and a photoperiod of 16:8 (L: D) for another 10 days to obtain cohorts of whitefly nymphs. After this period, the excluded nymphs were used in the bioassays or offered to the parasitoid to obtain pupae of the parasitoid in second nymphal instar, the stage which is susceptible to fungus and suitable to the parasitoid [28, 31].

2.2.3. Bioassay of treatments on eggs and second instar nymphs of B. tabaci

In the bioassays, Muniz and Nombela`s method [32] was followed. Pretests were performed using serial dilutions of ZnO NPs, Kaolin and NeemAzal-T/S to determine concentrations caused 10 to 90 percent mortality in exposed eggs and nymphs of the pest. The solutions were shaken for 10 min by a mechanical shaker before use. Afterward, the leaves bearing eggs and nymphs of the pest were dipped in ZnO NPs (3.5, 6, 12, 18 and 22 mg L^{-1}), Kaolin (20, 25, 30, 35 and 40 g L^{-1}) and NeemAzal-T/S (5, 10, 15, 25 and 50 mg AZA L^{-1}) for 5 s. Control leaves were dipped in distilled water. The leaves were allowed to dry at room temperature for 10 min and then the potted plants, kept at 24±2°C, 60%±5% RH, and a photoperiod of 16: 8 h L:D. The number of dead eggs (non-hatched) and nymphs were counted on 3rd day after treatment under a binocular. A nymph was considered dead if it was shrunken and lost its normal yellow-green color [33]. All experiments were performed with three replication using 20 eggs or nymphs (on potted cotton) in each replication. On each leaf only 10 eggs or 10 nymphs of the pest were remained and all others were removed.

2.2.4. Bioassay of treatments on pupae of the parasitoid

Groups of 5-6 females and 2-3 males of the parasitoid were collected with an aspirator from the colony and released to the clip cages where placed on the labeled cotton leaves as described before and allowed to parasitize the second instar nymphs of the pest for 24 h. Then the parasitoids were removed and leaves with parasitized nymphs incubated for another 11 days under similar conditions as above until pupation of the parasitoid (parasitoid pupae with the cherry colored eyes became visible through the whitefly cuticle).

Afterwards, these leaves were dipped in the same concentrations of ZnO NPs, Kaolin and NeemAzal-T/S as described before. In the control, the same procedure was performed but the leaves were dipped in distilled water. The number of empty pupal and pupae that failed to emerge was counted on 3th and 7th day after treatment. The experiment was conducted using 60 pupae of the parasitoid in three replication, 20 pupae in each replication.

2.2.5. Effects of treatment on the pest and parasitoid

An identical procedure as above was performed to evaluate the effects of ZnO NPs, Kaolin and NeemAzal-T/S alone or in pairwise combination on the eggs and nymphs of the pest as well pupae of the parasitoid. Based on the results of bioassays, eggs and second instar nymphs of *B. tabaci* and pupae of the parasitoid were exposed to different treatments as bellow:

ZnO NPs (LC_{50}), Kaolin (LC_{50}), NeemAzal (LC_{50}), NeemAzal (LC_{25}) + ZnO (LC_{25}), NeemAzal (LC_{25}) + Kaolin (LC_{25}), Control (distilled water). The mortality of the exposed eggs and nymphs was counted in each treatment in 3 and 7 days after treatment. All experiments were performed with 3 replications as in the bioassays.

2.3. Field experiments

2.3.1 Field planting

Experimental plots were prepared by disking after deep plowing. 150 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5 and 50 $kg.ha^{-1}$ K_2O used. Seeds of *Gossypium hirsutum* L., cv. *Varamin*, a susceptible cultivar to *B. tabaci* were planted in the research field of scientific staff of Cotton Research Center of East Iran, Kashmar (35° 13′ N, 58° 26′ E), in a randomized complete block design (RCBD) with three replications in August 2019. The experimental plots were 6 m^2 and 1.5 m apart with a distance of 70 cm between rows and 20 cm between plants after thinning. The blocks were 2 m apart. Since adult of whiteflies were very mobile and might enter into the treated plots, the control plots were located 30 m apart from treated plots. Handpicking of weeds was performed twice when the cotton was in four and eight leaf stages. The farm was irrigated every two weeks. An insecticide (Imidacloprid SC 35% EXIR, Iran) was used to control aphids and trips, 250 mL ha^1 when the cotton was in two leaf stage.

2.3.2. Estimating pest population

The pest population was estimated prior to the trials by counting eggs and nymphs of the pest and pupae of the parasitoid. For this purpose, 5 plants were randomly selected in each plot, 3 leaves from each one (top, middle and bottom section of plant canopy) were excised and transferred to the laboratory inside plastic bags (= altogether 45 leaves per treatment). Two pieces of 1 cm^2 from each leaf were chosen so that the units included the main vein (altogether 90 sample units per treatment). Sampling was made 0, 3, 7 and 14 day after treatment. The total live eggs and second instar nymphs of the pest as well pupae of the parasitoid in each sample unit were counted using a binocular. Samplings were carried out 1 day before and 3, 7 and 14 days after spraying.

2.3.3. Estimating parasitoid population

Same procedures used to estimate pest population were used to estimate population of the parasitoid, using the same sample units, by counting the pupae of the parasitoid formed in the nymphs of the pest and incubating all them at the experiment conditions for 5 days.

2.3.4. Efficacy of the treatments in field conditions

Recommended dose of NeemAzal-T/S (15 mg L^{-1} AZA), Recommended dose of Kaolin (30 g L^{-1}), a concentration of 20 mg L^{-1} of ZnO NPs (= LC_{75} and LC_{78} for egg and nymph, respectively based on bioassay), a dose of NeemAzal-T/S (7.5 mg L^{-1} AZA) + 10 mg L^{-1} of ZnO NPs and a dose of NeemAzal-T/S (7.5 mg L^{-1} AZA) + 15 g L^{-1} of Kaolin were prepared and sprayed on upper and lower surfaces of the cotton plants using a knapsack

sprayer (16 L capacity) at 300 mL per plot, equivalent to an application volume of 500 L ha^{-1}. Control treatment was sprayed with water only. Mortality produced by each treatment on the egg or nymphs of *B. tabaci* and pupal stage of *E. mundus* was calculated using the Henderson and Tilton equation (1) which corrects for the underlying natural mortality recorded in the controls [34]:

$$\text{Efficacy of treatment (\%)} = 1 - \left(\frac{\text{Ta}\times\text{Cb}}{\text{Ca}\times\text{Tb}}\right) \times 100 \quad (1)$$

where T= number of live egg, nymph or pupae per 5 plants in treatment after (Ta) or before (Tb) application, and C= number of live egg, nymph or pupae per 5 plants in control after (Ca) or before (Cb) application.

2.3.5. Analysis of data

The values of treatment efficacy calculated by the Henderson and Tilton formula from all three blocks were subjected to analysis. A general linear model (GLM procedure in SPSS) was used to compare the efficacy of the treatments in field conditions. In all trials; eggs, nymphs and pupae were recorded as dead or alive. In the case of non-homogeneity, percent values were transformed using arcsine-square-root (Arcsin√) transformation. One-way ANOVA (SPSS v.18.0; SPSS, Chicago, IL, USA) was used to analyze the mortality of treatment on eggs and nymphs of whiteflies or pupae of parasitoids. Means were compared by Tukey's Studentized Range Test, $P<0.05$.

3. Results and Discussion

The LC_{25} and LC_{50} values of ZnO NPs, Kaolin and NeemAzal-T/S on eggs and nymphs of the pest B. tabaci as well on the pupae of the parasitoid *E. mundus* were presented in tables 1.

Table 1. Probit analysis data for egg and second instar nymph of *B. tabaci* and pupae of *E. mundus* treated with ZnO NPs, Kaolin and NeemAzal-T/S on 3th day after treatment.

Treatment	Stage	LC_{50} (95% CL)	LC_{25} (95% CL)	Intercept±SE	Slope±SE	X^2
				Pest		
ZnO		7.49 mg L^{-1} (6.64-8.35)	2.71 mg L^{-1} (2.09-3.30)	3.66±0.12	1.52±0.12	15.25
Kaolin	Egg	24.89 g L^{-1} (23.53-26.09)	16.29 g L^{-1} (14.25-17.93)	0.11±0.49	3.66±0.33	12.37
NeemAzal (a.i.)		6.83 mg L^{-1} (5.43-8.15)	1.77 mg L^{-1} (1.07-2.51)	5.19±0.04	1.14±0.10	15.43
ZnO		6.93 mg L^{-1} (6.17-7.71)	2.50 mg L^{-1} (1.95-3.02)	3.72±0.11	1.52±0.11	12.01
Kaolin	Nymph	18.36 g L^{-1} (17.32-19.44)	10.57 g L^{-1} (9.38-11.60)	1.45±0.28	2.81±0.21	12.99
NeemAzal (a.i.)		6.00 mg L^{-1} (4.50-7.40)	1.30 mg L^{-1} (0.70-2.00)	5.23±0.04	1.04±0.10	19.64
				Parasitoid		
ZnO		11.30 mg L^{-1} (10.17-12.46)	4.34 mg/L (3.40-5.19)	3.29±0.15	1.62±0.13	20.94
Kaolin	Pupa	41.59 g L^{-1} (38.37-46.59)	22.91 g L^{-1} (20.57-24.76)	0.79±0.44	2.60±0.29	15.65
NeemAzal (a.i.)		36.90 mg L^{-1} (31.11-44.28)	6.87 mg L^{-1} (4.51-9.25)	4.47±0.05	0.92±0.09	21.17

3.1. Mortality of the eggs and nymphs of the pest in laboratory conditions

The mortality of eggs of the pest in laboratory conditions was different in all treatments both in 3 DAT ($F_{5,\ 12}$ = 233.67, $P<0.001$) and 7 DAT ($F_{5,\ 12}$ = 228.64, $P<0.001$), so that in 3 DAT and 7 DAT of experiment, the highest and lowest percentage of mortality was observed in Kaolin + NeemAzal and control, respectively (Figure 1).

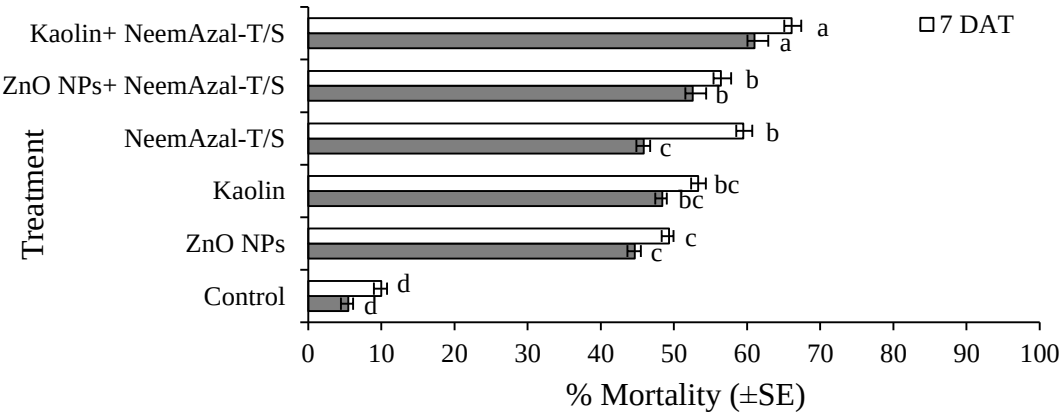

Figure 1. Mean (± SE) mortality (%) of different treatments in controlling eggs of *B. tabaci* on cotton applied either alone (LC_{50}) or in pairwise combination (LC_{25} + LC_{25}), measured 3 and 7 days after treatment (DAT). Means marked with different letters within the same column are significantly different ($P < 0.05$, Tukey).

As shown in figure 2, all treatments were different in both 3 and 7 DAT ($F_{5, 12} = 597.27$, $P<0.001$ and $F_{5, 12} = 242.65$, $P<0.001$, respectively). The highest mortality of nymphs of the pest at 3 DAT, was observed in Kaolin + NeemAzal (= ZnO+ NeemAzal), while the control had the lowest mortality. At 7 DAT, the highest mortality was observed in NeemAzal which was not different with ZnO+ NeemAzal and Kaolin + NeemAzal, while the lowest efficacy was observed in control. At both 3 and 7 DAT, ZnO + NeemAzal and Kaolin + NeemAzal exerted a higher level of control on nymphs of the pest than either ZnO or Kaolin.

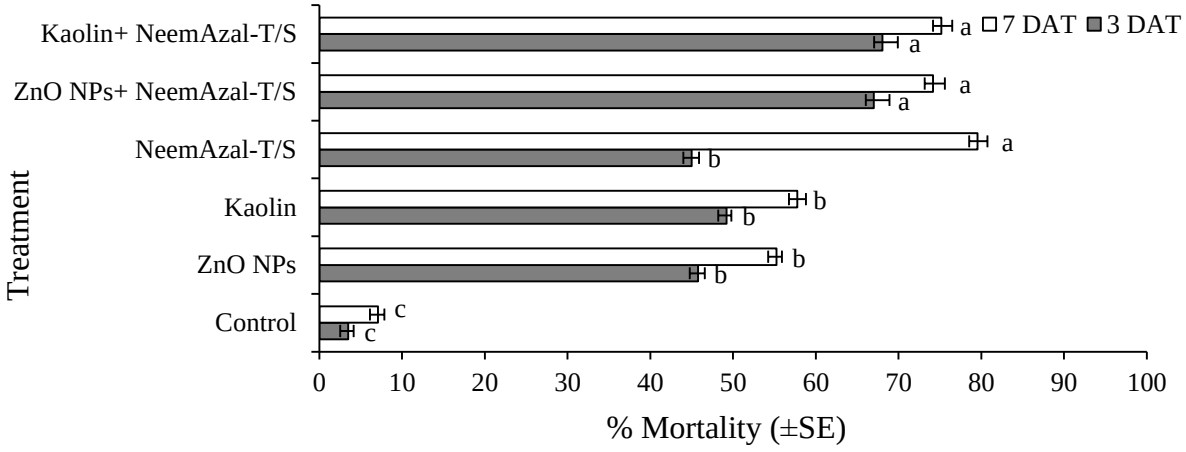

Figure 2. Mean (± SE) mortality (%) of different treatments in controlling nymphs of *B. tabaci* on cotton applied either alone (LC_{50}) or in pairwise combination (LC_{25} + LC_{25}), measured 3 and 7 days after treatment (DAT). Means marked with different letters within the same column are significantly different ($P < 0.05$, Tukey).

3.2. Mortality of the pupae of the parasitoid caused by different treatments in laboratory conditions

Effects of all treatments on the pupae of the parasitoid were different both in 3 DAT ($F_{5, 12} = 239.89$, $P<0.001$) and 7 DAT ($F_{5, 12} = 132.23$, $P<0.001$). At 3 DAT and 7 DAT, Kaolin + NeemAzal and followed by ZnO+ NeemAzal exerted a lower mortality on pupae of the parasitoid than either alone, while the control had the lowest mortality (Figure 3).

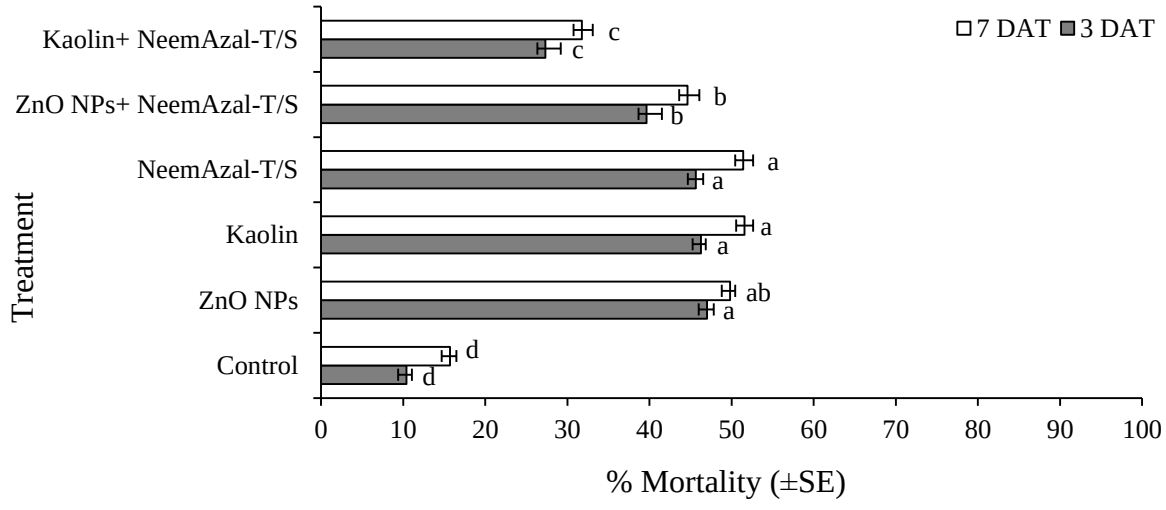

Figure 3. Mean (±SE) effect mortality (%) of different treatments on pupae of the parasitoid *E. mundus* parasitized nymphs of *B. tabaci* on cotton applied either alone (LC_{50}) or in pairwise combination (LC_{25}+LC_{25}), measured 3 and 7 days after treatment (DAT). Means marked with different letters within the same column are significantly different ($P < 0.05$, Tukey).

3.3. Efficacy of treatments in field conditions

Table 2 presented the mortality of eggs of the pest, caused by different treatments at 3, 7 and 14 day after treatment. At 3 DAT, the highest and lowest efficacy was observed in Kaolin + NeemAzal and NeemAzal, respectively ($F_{4, 8} = 31.91$, $P<0.001$). The highest and lowest efficacy at both 7 and 14 DAT was observed in

Kaolin + NeemAzal and ZnO, respectively ($F_{4, 8} = 15.16$, $p = 0.001$ for 7 DAT and $F_{4, 8} = 37.04$, $P<0.001$ for 14 DAT).

Table 2. Mean (±SE) efficacy (%) of different treatments in controlling eggs of *B. tabaci* on cotton applied either alone or in pairwise combination, measured 3, 7, and 14 days after treatment (DAT) in field.

Treatment	Recommendation Rate	Mean (±SE)			
		3 DAT	7 DAT	14 DAT	Mean
ZnO NPs	20 mg L^{-1}	34.06±0.08 ^{bc}	14.82±0.05 ^{c}	1.77±0.07 ^{d}	16.88
Kaolin	30 g L^{-1}	44.32±0.08 ^{b}	46.53±1.31 ^{ab}	21.11±0.06 ^{b}	37.32
NeemAzal	15 mg L^{-1}	24.44±0.05 ^{c}	40.93±0.12 ^{b}	18.26±0.04 ^{bc}	27.87
ZnO NPs +NeemAzal	10 mg L^{-1} + 7.5 mg L^{-1}	38.52±0.06 ^{b}	32.34±0.08 ^{bc}	10.36±0.07 ^{cd}	27.07
Kaolin +NeemAzal	15 g L^{-1} + 7.5 mg L^{-1}	58.34±0.21 ^{a}	67.43±0.12 ^{a}	32.61±0.08 ^{a}	52.79

Means marked with different letters within the same column are significantly different (GLM Univariate followed by Tukey's test: $P<0.05$).

As shown in table 3, the efficacy of all treatments on controlling nymphs of the pest was different at 3, 7 and 14 day after treatment. The highest and lowest efficacy at 3 DAT was observed in Kaolin + NeemAzal and NeemAzal respectively ($F_{4, 8} = 29.26$, $P<0.001$). The highest and lowest efficacy at both 7 and 14 DAT was observed in NeemAzal and ZnO, respectively ($F_{4, 8} = 20.20$, $P<0.001$ for 7 DAT and $F_{4, 8} = 18.35$, $P<0.001$ for 14 DAT).

Table 3. Mean (±SE) efficacy (%) of different treatments in controlling nymphs of *B. tabaci* on cotton applied either alone or in pairwise combination, measured 3, 7, and 14 days after treatment (DAT) in field.

Treatment	Recommendation Rate	Mean (±SE)			
		3 DAT	7 DAT	14 DAT	Mean
ZnO NPs	20 mg L^{-1}	55.48±0.10 ^{b}	35.73±0.07 ^{b}	3.44±0.07 ^{c}	31.55
Kaolin	30 g L^{-1}	56.60±0.15 ^{b}	54.50±0.06 ^{b}	40.64±0.06 ^{ab}	50.58
NeemAzal	15 mg L^{-1}	45.45±0.09 ^{c}	86.52±0.11 ^{a}	46.39±0.10 ^{a}	59.45
ZnO NPs +NeemAzal	10 mg L^{-1} + 7.5 mg L^{-1}	68.34±0.07 ^{a}	77.43±0.08 ^{a}	21.31±0.06 ^{bc}	55.69
Kaolin +NeemAzal	15 g L^{-1} + 7.5 mg L^{-1}	70.56±0.07 ^{a}	78.66±0.08 ^{a}	40.87±0.05 ^{ab}	63.36

Means marked with different letters within the same column are significantly different (GLM Univariate followed by Tukey's test: $P<0.05$).

3.4. Mortality of the parasitoid pupae in different treatments in field conditions

The mortality caused by different treatments on pupae of the parasitoid were different at 3 DAT ($F_{4, 8} = 5.52$, $p = 0.02$), 7 DAT ($F_{4, 8} = 8.71$, $p = 0.005$) and 14 DAT ($F_{4, 8} = 10.89$, $P<0.003$) (Table 4). The highest and lowest mortality of parasitoid pupae at 3 DAT was observed in ZnO + NeemAzal and Kaolin respectively. The highest and lowest mortality of parasitoid pupae at 7 DAT, was occurred in NeemAzal and ZnO, respectively. The highest mortality at 14 DAT was observed at Kaolin and the lowest was occurred in ZnO (Table 4).

Table 4. Mean (±SE) effect mortality (%) of different treatments on pupae of the parasitoid *E. mundus* parasitized nymphs of *B. tabaci* on cotton, measured 3, 7, and 14 days after treatment (DAT) in field.

Treatment	Recommendation Rate	Mean (±SE)			
		3 DAT	7 DAT	14 DAT	Mean
ZnO NPs	20 mg L^{-1}	11.23±0.04 ^{ab}	2.17±0.04 ^{b}	0.00±0.00 ^{b}	4.46
Kaolin	30 g L^{-1}	3.19±0.06 ^{b}	13.21±0.11 ^{a}	6.40±1.02 ^{a}	7.60
NeemAzal	15 mg L^{-1}	10.50±0.06 ^{ab}	15.94±0.08 ^{a}	3.25±0.05 ^{ab}	9.89
ZnO NPs+NeemAzal	10 mg L^{-1} + 7.5 mg L^{-1}	13.76±0.07 ^{a}	9.41±0.06 ^{ab}	0.72±0.09 ^{b}	7.96
Kaolin +NeemAzal	15 g L^{-1} + 7.5 mg L^{-1}	6.24±0.09 ^{ab}	12.11±0.08 ^{a}	5.31±0.25 ^{a}	7.88

Means marked with different letters within the same column are significantly different (GLM Univariate followed by Tukey's test: $P<0.05$).

In present study, the LC_{50} values for ZnO NPs on *B. tabaci* nymphs and eggs were ranges between 6.93 mg L^{-1} and 7.49 mg L^{-1}, respectively. In a similar study, Khooshe-Bast et al. [35] investigated the insecticidal effects of ZnO NPs on *Trialeurodes vaporariorum* adults and found out the LC_{50} and LC_{25} values were 7.35 mg L^{-1} and 3.76 mg L^{-1}, respectively. Our results demonstrated high levels of *B. tabaci* nymphs control efficacy with combinations of NeemAzal-T/S and either ZnO NP or Kaolin. It appears that the toxicity of ZnO against whitefly nymphs possibly enabled by the small size of its nanoparticles, which could passage easily through the insect cuticle and enter into the individual cells where they interfere with molting and other physiological processes [12]. Increased exposed surfaces of ZnO which could interact with the insect cuticle, could play role in toxicity of this nanoparticle. Mortality percentages of egg and nymphs of the pest in present study in controls were similar to those reported by Khan and Wan [36]. In present study, 46.53 % mortality of eggs of *B. tabaci*, caused by Kaolin treatment at 7 day after treatment in field. Similarly, Bestete et al. [37] reported that fewer sap-sucking whiteflies (*B. tabaci*) under choice trial colonized kaolin treated plants, and they laid approximately 50 % fewer eggs on these cotton plants. In the laboratory, Liang and Liu [38] also found fewer adults and oviposition of *Bemisia argentifolii* Bellows and Perring (Hemiptera: Aleyrodidae) on melon (*Cucumis melo* L.) leaves sprayed with kaolin. No differences were found between the Kaolin and Kaolin +NeemAzal treatments for efficacy of nymph reduction at 14 day after treatment. Núñez-López et al. [39] also found higher than 60% efficacy in the control of nymphs and eggs of *T. vaporariorum* due to the foliar applications of kaolin; they concluded that the use of kaolin can be considered an alternative in integrated pest management because its efficacy can be equal to that of chemically synthesized insecticides, and its effects on plant physiology are positive. Kaolin is suggested to affect the cuticular permeability [40] to water which is one of the limiting factors for insects. Moreover, Kaolin on plants may prevent pests from identifying a host, and pest activity can also be impaired by the kaolin particles that stick to their bodies [41, 42]. Previous studies found that foliar Kaolin sprays reduced the adults and nymphs of *Agonoscena targionii* (Hemiptera: Psyllidae) in pistachio by 80% [43]; the number of eggs and nymphs of *Diaphorina citri* (Hemiptera: Liviidae) on citrus by 85% and 80% [44] and the nymphs population of *Cacopsylla pyri* (Psyllidae) on pear trees by 85% [45]. The pupae of *E. mundus*, such as eggs and nymphs of *B. tabaci*, probably are vulnerable to desiccation. Kaolin affects the cuticle by removal or sorption of cuticular waxes, causing a loss of water from the body, and subsequently death through desiccation [44]. The highest mortality of parasitoid pupae at 7 DAT was observed in Kaolin treatment. Similarly, kaolin treated surface caused a slight reduction in the parasitism of *Psyttalia concolor* (Szepligeti) (Hymenoptera: Braconidae) in a behavioral dual choice experiment; but there was no differences in a no choice experiment on the percentage of mortality and attacked hosts [46].

In our study, the highest egg mortality (40.93%) was observed for the field application rates of NeemAzal-T/S at 7 DAT. Kumar and Poehling [29, 47] suggested that egg hatch failure is due to the penetration of azadirachtin solution sprayed onto the leaves. Also, ingestion of azadirachtin from plant tissues by females could inhibit of embryonic development or the hatching process, and be caused an intrinsic deficit of the eggs deposited by females. Antifeedant and deterrent actions of neem resulting in decreased egg deposition by *B. tabaci* have also been reported in earlier studies [28]. Eggs are usually regarded less susceptible to insecticides compared to other stages. The apparent reduction in nymphs for NeemAzal maybe occurred due to the effect of NeemAzal on crawlers after eclosion from viable eggs when they came into contact with the residues on leaves. Again, pupal stage is usually regarded more resistant to insecticides and other mortality factors. This could be due to the presence of thick cuticular layers which protect the pupa from any contact to toxicant materials. Kumar et al. [48] reported that using the recommended dose rates of NeemAzal (50 mg a.i. L^{-1}) on *Eretmocerus warrae*, a parasitoid of *B. tabaci* caused emergence rate of 55% at pupal stage. Because the *E. mundus* larva penetrates its host by chewing a hole in the host cuticle it opens a path for the topically applied biopesticide solution to enter the body. Hence the whitefly as well as the parasitoid inside can be contaminated directly with the active ingredient, which is a kind of worst case situation. For that reason these ecto-endo aphelinid parasitoid species are more susceptible to the foliar application of neem than exclusively endoparasitic species [49]. In present study, the overall control of whitefly with NeemAzal was satisfactory. Lower efficacy of NeemAzal on eggs and nymphs, 14 days after treatment, compared with 7 DAT maybe due to exposure to sunlight and environmental conditions, resulting in faster degradation of NeemAzal in the field conditions. The major problem with neem-based products, which have triterpenoids as the active ingredient, is the photo-degradation by UV radiation [50, 51]. This reduction in bio-efficacy of neem seems to be related to the amount entering into the plant system, to exposure to sunlight and climate conditions [51].

4. Conclusion

In conclusion, due to satisfactory level of control of the ZnO NPs, Kaolin clay and NeemAzal-T/S, on eggs and nymphs of the *B. tabaci* both alone or in combination form in field conditions, they could be recommended to use in IPM of this pest on cotton, although they exerted a low level of mortality on the parasitoid pupae.

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