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Research Article

Laboratory evaluation of *Aschersonia aleyrodis* (Webber) against citrus blackfly, *Aleurocanthus woglumi* (Ashby) and whitefly, *Dialeurodes citri* (Ashmead)

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ABSTRACT: Red whitefly fungus, *Aschersonia aleyrodis* (Webber) is a fungal pathogen of nymphs of blackfly, *Aleurocanthus woglumi* (Ashby) and whitefly, *Dialeurodes citri* (Ashmead) on the citrus plant. It is used as a promising biological control agent. In the present study, attempts have been made to check the efficacy of *A. aleyrodis* biopesticides for the management of citrus blackfly and whitefly under laboratory condition. Probit analysis for LC50 values of 2nd instar nymph was calculated 1.1×10^8 spore/ml and 1.8×10^7 spore/ml and LT50 values, 8.14 to 13.77 days and 6.88 to 9.72 days were derived for 2nd instar nymph of citrus blackfly and whitefly at respective concentration. Cross infectivity was found non-pathogenic to citrus aphids and mealybugs. Among the insecticides, Dimethoate 30 % EC was observed compatible with *A. aleyrodis* as it shows less inhibition at the half and recommended dose of insecticide.

KEYWORDS: *Aschersonia aleyrodis*, *Aleurocanthus woglumi,* biological control, entomopathogenic fungus

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INTRODUCTION

Amongst the fruit crops, citrus (family – Rutaceae) occupies the third position with respect to area and production in India. It is a globally cultivated fruit crop, which includes Mandarin orange, Acid lime and other related species of citrus. Citrus fruits are rich in vitamin C, minerals and alkaline salts. Insect and mite infestation results in the loss of more than 30% of the nation's citrus production every year (Aruna *et al*., 2017). Citrus blackfly, *Aleurocanthus woglumi* (Ashby) and whitefly, *Dialeurodes Citri* (Ashmead) are considered the most injurious insects infesting citrus trees right from the nursery stage to the harvesting (Ingle *et al*., 2019). Management of infestations is very difficult due to the hard coating on their body. However, injudicious use and overuse of synthetic chemicals have resulted in the development of pest resistance to pesticides. Apart from this, chemicals cause harmful effects on non-target organisms and lead to the development of environmental hazards which demands the use of alternate strategy for the control of blackflies and whitefly in the citrus crop. Biological control methods are found to be the best alternative method.

Aschersonia aleyrodis on citrus plantations were used in Florida from the early 1900's as a classic biological control agent against *Dialeurodes citri*. Whitefly pests are considered to be under virtually complete control by the use of *A. aleyrodis* in Florida. Similarly, between 1958 and 1973 strains of several species of *Aschersonia* were introduced from China, India, Japan, Vietnam, the U.S.A. and Cuba for the use of biological control of the citrus whitefly. Under favourable environmental conditions, this fungus shows approximately 80% of larval mortality and adapted well to new citrus plantations (Ingle *et al.,* 2022).

The present study has been made to find out the efficacy of new biopesticides for the management of citrus black flies and whiteflies under laboratory conditions. Combined use of entomopathogenic fungi and sublethal doses of chemical insecticide has also been examined to determine the possible usefulness of combinations against citrus blackfly and whitefly which will be helpful for integrated pest management programs.

MATERIALS AND METHODS

Rearing of test insect

Blackfly (*A. woglumi*) and whitefly (*D. citri*) were used as test insects for assessing the pathogenicity of *A. aleyrodis.* The naturally heavy infected nymph of Citrus Blackfly and Citrus Whitefly seedlings of acid lime (planted in polybag) are separated out from the nursery and kept under a wooden chamber having a facility for aeration and protection from other insects. The rearing conditions were set at room temperature and a photoperiod of 12 hours of light and 12 hours of darkness.

Preliminary test for bioassay

Citrus blackfly (*A. woglumi*) and whitefly (*D. citri*) were used as test insects for assessing pathogenicity. For deciding the hypothetical concentration, the preliminary trial having a concentration of 10^{10}, 10^9, 10^8, 10^7, 10^6, 10^5, and 10^4 spores/ml of a fungal suspension of *A. aleyrodis* was tested against whitefly and blackfly second instar nymph. The nymphal mortality was recorded on the 10th day after treatment. Based on mortality ranging between 30-90 % the concentration was decided for the final testing. In the preliminary studies, among the concentration 10^5 to 10^9 spores /ml was selected, as they recorded 30-90 % mortality.

Figure 1. Naturally infected population of citrus blackfly collected from Morshi Tahsil of Amravati District.

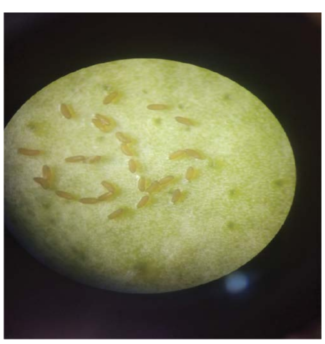

Figure 2. Citrus blackfly eggs under microscope.

Final testing

Bioassay was carried out as per the procedure given by Zhang *et al.,* (2016). Concentrations of fungal spores (as spore basis) i.e. 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 spores/ml were obtained by serial dilution method. Acid lime seedlings having Blackfly and Whitefly nymph populations were placed in bioassay cages to exclude foreign infestations from other insects before being transferred, the cage house was thoroughly disinfected to exclude any foreign microbes and insects that might be harboured in the cage house.

Ten seedlings of acid lime having enough population of second instar nymph of whitefly and blackfly was sprayed with 1 ml each of *A. aleyrodis* respective concentration and control were treated with 0.05% Tween 80 solution. The moist condition was maintained in the cage house by watering seedlings and keeping water-soaked cloth for 5 days. After five days of treatment, the treated leaf of respective concentrations of whitefly and blackfly detached and counted the nymphal population of test insects and placed in 9.0 cm Petri dishes with moistened filter paper laid at the bottom of Petri dishes. A small amount of water was added daily to prevent desiccation and the dishes were inoculated under the standard experimental conditions of 25 ± 2°C and 75 ± 5% RH. The mortality was noted at every 24 hours up to 15 days and the experiment was replicated five times.

The mycosis insects are counted as dead and the percent mortality and time required to kill the insect in days were calculated. The cumulative mean percent mortality obtained on the 15th day was considered for bioassay studies. These mortality percentages were then corrected by using Abbotts's formula.

$$\text{Correct mortality} = \frac{\text{T-C}}{\text{100-C}}$$

Where, T = % mortality in treatment
C = % mortality in control

Evaluation of cross infectivity against other sucking pest

Sucking pest viz. Citrus aphid and Mealybugs were collected from citrus orchards. Pure fungal culture of *A. aleyrodis* was suspended in 0.05% tween 80 and vortexed for 1 minute to produce a homogenous suspension. The conidial suspension was then diluted with sterile distilled water made concentration of 10^8 spores/ml by serial dilution and topically applied on mealy bug citrus aphid and cotton aphid by hand atomizer. After treatment, seedlings are kept in moist conditions under observation. The insects infected with *A. aleyrodis* were considered dead when there was a white fringe around the insect's body or it had turned white or orange. Only insects that had died due to test fungus infection were counted in the mortality calculation.

Figure 3. Symptoms produced by *A. aleyrodis* on blackfly and whitefly populations.

Table 1. List of insecticides used

Sr. No.	Chemical name	Trade name	Company	Conc. (%)	
				RD	HD
1	Imidacloprid17.8% SC	Bildor	Bharat insecticide	0.1	0.05
2	Dimethoate 30% EC	Rogar	Gayatri Pesticides Private Ltd.	0.1	0.05
3	Quinolphos 25% EC	Krush	Biostadt Ltd.	0.2	0.1
4	Chloropyriphos 20%EC	Rickcare	Darrick Insecticides Ltd.	0.1	0.05
5	Thiamethoxam 25%WG	Pele	Bharat insecticide	0.1	0.05
6	Dicofol 18.5% EC	Colonel-s	Indofil Industries Ltd.	0.2	0.1
7	Fipronil 5% SC	Agadi	Adama India Pvt Ltd	0.25	0.125

RD: Recommended Dose, HD: Half Dose

Compatibility of *A. aleyrodis* with different insecticides

Following insecticides were tested at two levels i.e. recommended dose, and half of the recommended dose. For *in vitro* evaluation of chemical insecticides for which poison food technique was adopted.

The required quantity of insecticides was taken with the help of a micropipette and transferred into autoclaved molten media in plates that were inoculated centrally with a 4 mm disc of young sporulating culture of *A. aleyrodis* at 25 ± 1 °C in the incubator. The percent inhibition of mycelial growth was calculated with the formula given below.

$$I = \frac{C-T}{C} \times 100$$

Where, C = Growth of fungus in control (mm) after 10 days of incubation

T = Growth of fungus in treatment (mm) after 10 days of incubation

I = % Growth inhibition after 10 days of incubation

Statistical analysis

The Log Dose Probit (LDP) bioassay was carried out to work out the median Lethal Concentration (LC50) and median Lethal Time (LT50). The mortality data were subjected to probit analysis as per the procedure of Finney (1964) and also by using computer software PROBITUSK.

RESULTS AND DISCUSSIONS

Susceptibility test

Pathogenic abilities of *A. aleyrodis* at 10^8 spore/ml concentration were assessed against the second instar nymph of citrus blackfly and whitefly by a direct spray of spore suspension on the body of the insect. The nymphal mortality was recorded for up to 15 days and cumulative mean percent larval mortality was calculated. Cultured *A. aleyrodis* isolate was found pathogenic to the tested insect. Early infection of fungus on the blackfly and whitefly nymph was usually detected as the same white fringes of hyphae extending from the marginal area of the nymphal body and as pustule at the weak points on the insect dorsum. At the end of the infection, mat-like pustules were observed completely covering the nymphal body. Colonies were white at first and later became yellowish to orange in colour as sporulation began.

Dose mortality response of citrus blackfly against *Aschersonia aleyrodis*

The bioassay studies against citrus black flies showed varied degrees of mortality concerning time. The results of the bioassay of *A. aleyrodis* consisting of five concentrations i.e. 10^5, 10^6,10^7, 10^8, 10^9 spores/ml the results are presented in Table 2 revealing the potency of *A. aleyrodis* against the citrus blackfly. The LC50 values for all the concentration is 1.1×10^8 spores/ml and the upper and lower fiducial limit is 1.8×10^7- 7.5×10^8 spores/ml.

The percent nymphal mortality ranged between 26.66 to 73.33% for different concentrations (Table 3). No larval mortality was observed in the control. Median lethal time ranges between 11.57 to 14.77 days. Among the respective concentration, the least LT50 value was observed in 1×10^9 spore/ml concentration i.e. 11.57 days with 11.00 to 12.26 days fiducial limit followed by 1×10^8 spore/ml concentration i.e. LT50 value 12.81 days. The maximum LT50 value was observed at 1×10^5 spore/ml concentration. Among the five different concentrations, the values indicated the trend of $1 \times 10^5 < 1 \times 10^6 < 1 \times 10^7 < 1 \times 10^8 < 1 \times 10^9$. As the concentration increases the median lethal time decreases.

Dose mortality response of whitefly against *Aschersonia aleyrodis*

The results of bioassay of *Aschersonia aleyrodis* consist of five concentrations i.e., 10^5, 10^6, 10^7, 10^8, 10^9 spores/ml. Results presented in Table 4 indicate the potency of *A. aleyrodis* against the Whitefly the LC50 values for all the concentration is 1.8×10^7 spores/ml and the upper and lower fiducial limit is 1.8×10^6 - 6.9×10^7 spores/ml.

The results given in Table 5, reveal that the median Lethal Time ranges between 8.14 to 13.77 days. The lowest LT50 value was observed in 1×10^9 spore/ml concentration i.e. 8.14 days with a fiducial limit of 7.59 to 8.71 days. The relatively higher time required to kill whitefly in 1×10^8 spore/ml concentration i.e. 8.29 days has a 7.57 to 9.25 fiducial limit. The highest LT50 values were observed at a lower dose (1×10^5 spore/ml) i.e. 13.77 days having a fiducial limit of 11.55 to 21.20 days. Among the five different concentrations, the values indicated the trend of $1 \times 10^5 < 1 \times 10^6 < 1 \times 10^7 < 1 \times 10^8 < 1 \times 10^9$. As the concentration increases the median Lethal Time decreases. Findings are in agreement with results obtained by Zhang *et al.,* (2017) who stated that percent larval mortality depends on the age of blackflies and whitefly and the concentration of entomopathogenic fungus. *A. aleyrodis* showed high pathogenicity against second and third instars and pupae with LC50 values of 7.93×10^6, 1.08×10^7 and 1.56×10^7 conidia/ml respectively. The median Lethal Time (LT50) was lower (4.60 days) for second instars and was highest (6.17 days) for pupae when inoculated with concentrations of 1×10^7 conidia/ml. Pena *et al.,* (2009) also proved the pathogenicity of *A. aleyrodis* by topical application on citrus black flies and observed considerable mortality. Lima *et al.,* (2015) observed 65, 84, 42 and 25 % mortality rates in 1st, 2nd, 3rd and 4th instar citrus blackfly nymphs respectively. The percent mortality levels were not comparable exactly with earlier reports which might be due to the concentration, difference in strains and local tests insect culture used but they are in line with present findings.

Evaluation of cross infectivity of *Aschersonia aleyrodis* against sucking pests of citrus

For evaluation of cross infectivity against the other pest of citrus concentration of 1×10^8 spore/ml solution was tested on the citrus pests which were collected from the vicinity and treated in the laboratory.

The data given in Table 6 shows no mortality and fungal growth observed on the insect body when observed under the microscope. This was concluded that *A. aleyrodis* was nonpathogenic to citrus aphids (*T. citircidi*) and mealy bugs (*P. citri)* Fransen (1987) and Meekes *et al*., (2002) reported that *A. aleyrodis* fungus is host specific mainly confined pathogenic or parasitic to the family Aleyrodidae and Coccidae.

Table 2. Dose mortality response of second instar nymphs of citrus blackfly against *Aschersonia aleyrodis*

Sr. No.	Dose (spores/ml)	LC50 value (spores/ml)	Fiducial limit (upper and lower)	Slope ±SE	X^2
1	1×10^5	1.1×10^8	1.8×10^7-7.5×10^8	0.29±0.07	7.815
2	1×10^6				
3	1×10^7				
4	1×10^8				
5	1×10^9				

Table 3. Time mortality response of second instar nymphs of citrus blackfly against *Aschersonia aleyrodis*

Sr. No.	Dose (spore/ml)	Mean nymphal mortality (%)	LT50 value (days)	(Fiducial limit Upper and Lower)	Slope ±SE
1	1×10^5	26.66	14.77	13.29-26.41	10.23±3.43
2	1×10^6	40.00	14.53	13.58-17.18	10.59 ±2.6
3	1×10^7	46.66	13.92	13.58- 15.59	11.18±2.5
4	1×10^8	60.00	12.81	12.17-13.85	9.30±1.7
5	1×10^9	73.33	11.57	11.00 -12.26	8.5±1.28

Table 4. Dose mortality response of whitefly against *Aschersonia aleyrodis*

Sr. No.	Dose (spores/ml)	LC50 value (spores/ml)	Fiducial limit (upper and lower)	Slope ±SE	X^2
1	1×10^5	1.8×10^7	1.8×10^6 - 6.9×10^7	0.3733	7.815
2	1×10^6				
3	1×10^7				
4	1×1^08				
5	1×10^9				

Compatibility of *A. aleyrodis* with different chemical insecticides

Compatibility of *A. aleyrodis* with commonly recommended insecticides is necessitated in the context of Integrated Pest Management (IPM) which advocates the use of microbial insecticides and need-based application of pesticides. The experiment has proved that the insecticides Fipronil, Imidacloprid, Dicofol and Dimethoate were minimum inhibitory to *A. aleyrodis* as they exhibited 7.77, 7.38, 7.0 and 6.63 colony growth and per cent inhibition 13.70, 17.96, 22.19 and 26.37 % respectively, at half dose (Table 7). The rest of the insecticides like Quinolphos, Chloropyriphos and Thiomethoxam found quite inhibitory at half dose. At the recommended dose, the insecticide treatment Dimethoate significantly supported the maximum growth (5.2 cm) of *A. aleyrodis* over the rest of the insecticides. Other insecticides were found deleterious to the mycopathogen as recorded more than 50% inhibition at the recommended dose. Results of the present investigation revealed that the insecticides Imidacloprid, Dimethoate, Fipronil and Dicofol can be safely applied in the field along with half dose to reduce insect pests as they retained less toxic effect against *A. aleyrodis.* The findings revealed that the Dimethoate was safe to *A. aleyrodis* at half dose and recommended dose. These findings would be useful in the formation of IPM modules for the management of citrus black flies and white flies by using *A. aleyrodis.* Similar kind of results was also reported by Neves *et al*., (2001) who reported that Imidacloprid with three various concentrations was least toxic to *Beauvaria bassiana*, *Metarhizium anisopliae* and *Paecilomyces.*

Table 5. Time mortality response of whitefly against *Aschersonia aleyrodis*

Sr. No.	Dose	Mean nymphal mortality (%)	LT50 (days)	Fiducial limit (upper and lower)	Slope
1	1×10^5	33.33	13.77	11.55-21.20	5.07±3.43
2	1×10^6	46.66	10.65	9.55-13.09	5.71±2.89
3	1×10^7	56.66	10.70	9.88-12.09	4.71±2.75
4	1×10^8	73.33	8.29	7.57-9.25	4.38±1.83
5	1×10^9	86.66	8.14	7.59-8.71	5.48±1.43

Table 6. Evaluation of cross infectivity of *Aschersonia aleyrodis* against sucking pests of citrus

Sr. No.	Name of insect	Scientific Name	No of insects taken	Percent mortality observed
1	Citrus aphid	*Toxoptera citricidi*	20	No mortality was observed
2	Mealy bug	*Planococcus citri*	20	

CONCLUSION

The present *in vitro* investigation suggests that, the *Aschersonia aleyrodis* is promising fungal species that has shown considerable virulence. LC50 and LT50 values for 2nd instar nymph as 1.1×10^8 spore/ml and 1.8×10^7 spore/ml and 8.14 to 13.77 days and 6.88 to 9.72 days for different species of blackfly and whiteflies. Among the insecticides, Dimethoate 30% EC was observed compatible as less inhibition was recorded at half and recommended dose. Further study required for field verification and confirmation of doses as well as compatibility of insecticides with entomogenous fungi, *Aschersonia aleyrodis*.

Table 7. Compatibility of *A. aleyrodis* with different chemical insecticides

Sr. No.	Insecticides	Concentrations		Half dose		Recommended dose	
		HD	RD	Mean radial mycelial growth (cm)*10 DAI	Mycelial inhibition (%)	Mean radial mycelial growth (cm)*10 DAI	Mycelial inhibition (%)
1	Imidacloprid 17.8%SL	0.05	0.1	7.38	17.96	3.7	59.44
2	Dimethoate 30%EC	0.05	0.1	6.63	26.37	5.2	41.85
3	Quinolphos 25%EC	0.1	0.2	3.57	60.37	1.9	79.44
4	Chloropyriphos 20%EC	0.05	0.1	5.37	40.37	2.8	68.52
5	Thiomethoxam 25%WG	0.05	0.1	3.88	56.85	1.5	83.15
6	Dicofol 18.5%EC	0.1	0.2	7.00	22.19	3.8	58.15
7	Fipronil 5 %SC	0.125	0.25	7.77	13.70	3.0	66.26
	F' test	-		Sig.		Sig.	
	SE(m)±	-		0.07		0.04	
	CD(P=0.01)	-		0.32		0.18	

*average of three replications *DAI days after inoculation

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