Madras Agric. J., 2019; doi:10.29321/MAJ 2019.000293

RESEARCH ARTICLE

Bioassay of Azadirachtin Nanofomulation Against *Bemisia tabaci,* the Vector of Mungbean Yellow Mosaic Virus

Shanmugapriya, S[1], Jeya Sundara Sharmila, D*[1], Karthikeyan, G[2] and Subramanian, K.S[1]
*[1]Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore – 641 003.
[2]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641 003.

Received : 04th June, 2019
Revised : 11th June, 2019
Accepted : 12th June, 2019

ABSTRACT

The greengram is highly susceptible to *Mungbean yellow mosaic virus*, (MYMV) which causes 90% yield loss. The MYMV is transmitted by whiteflies (*Bemisia tabaci*) in a persistent manner. Most of the management strategies are through chemical pesticides as vector control. It was hypothesized to control whiteflies with a good alternative to pesticides. In this study, Azadirachtin loaded in silica nano particle and *Annosquamosin* plant biomolecule were studied for its efficacy against *Bemissia tabaci.* Azadirachtin loaded in silica nanoparticles at 500ppm showed cent per cent mortality of adult *Bemisia tabaci* followed by 90% mortality in Annosquamosin extract at 5 hrs of incubation. Thus, Azadirachtin loaded in silica nanoparticles can be used as a better alternative to the highly toxic chemical pesticides.

Keywords: *Whiteflies, Azadirachtin, Annosquamosin,MYMV*

INTRODUCTION

India is the world's largest producer and consumer of greengram by producing 1.5 – 2.0 million tonnes from 3 - 4 million hectors cultivable land. It accounts for 70% of world's greengram production and 10 – 12% of total pulses. They are the rich source of proteins (20 – 25%), riboflavin, vitamin – C and thiamine. The crop is highly susceptible to various pest and diseases such as gram pod borer, aphids, stem fly, whiteflies, thrips and *Cercospora* leaf spot, powdery mildew, rust yellow mosaic disease, leaf crinkle disease respectively(AESA based IPM - greengram). Among them, the whiteflies (*Bemisiatabaci)* is the significantly important causing economic loss either by transmitting deadly viruses or through sucking the sap. It is capable of causing 30 – 70% yield loss depending on the growth stage of the crop at which infection takes place (Lal, 1985; Marimuthu et al. (1981)). It is a polyphagous pest feeding on 78 plant species (Lai & Pillai, 1982; Misra & Singh, 1929; Naresh & Nene, 1980; Nene, 1972, 1973; Trehan and Bagal (1944)) and belongs to the family *Aleyrodidae* and super family *Hemoptera*(Jadhav & Armes, 1996). It feeds on the sap by inserting the stylet into the sieve elements present in phloem cells by which it reduces the photosynthetic activity of foliage thereby reducing the nutrient content (Bethke et al., 1991). Apart from it, honey dew secretion on leaves will encourage scooty molds and ants which repeal the natural enemies controlling whiteflies (Markham et al., 1996; McAuslane, 2002). This results in the yellowing and wilting of leaves producing distorted or crinkled seeds (Chu et al., 1995; Fishpool & Burban, 1994). It is the vector for wide range viruses such as geminivirus, closterovirus, nepovirus, potyvirus, carlavirus and a rod-shaped DNA virus (Markham et al., 1996). *B.tabaci* is susceptible to parathion, baythion and malathion at 0.1% and monocrotophos at 0.05% (Nene, 1973). Aldicarb, phorate, carbofurandisulfoton are used in soil application at 1 – 2 kg of a.i/ ha. The continuous application of chemical compounds in the agricultural field increases the residual effect, resistance development, toxicity to non-target organisms and reduces the fertility of the soil (Bag, 2000). As per the census of World Health Organisation (WHO) pesticide causes poisoning and death of 2,00,000 peoples in a year (Yadav et al., 2015)(World Health Organisation, 1990).The noteworthy alternative to this synthetic pesticide is phytochemicals commonly called biopesticide because of its eco-friendly, biodegradability and low toxicity. It includes Azadirachtin from the neem tree, citronellal from lemongrass, turmurone from *curcuma longa.* Among which Azadirachtin from neem emerged as a major powerfullbiopesticide(Chaudhary et al., 2017). The effect of silica nano particles on the nymphal stage of whiteflies showed 91% of mortality (Debnath et al., 2011). It has a different mode of action such as antifeedant, disrupts ecdysis thereby preventing molting, prevents oogenesis and oviposition and reduces sperm production. Although Azadirachtin has added advantage over the synthetic pesticide,

*[1]Corresponding author's e-mail: djssharmila@gmail.com

its cost of production, risk in sustainable source for extraction , sensitivity towards UV radiation and leaching effect limited its utilisation(Pavelthe a, 2014). New formulation strategy for controlled and protected release of Azadirachtin – A from sodium alginate capsules was reported by (Flores-Céspedes et al., 2015). In the present work, the effect of silica based controlled delivery system against *B.tabaci*was studied.

MATERIAL AND METHODS

The *Bemisia tabaci*, the most efficient transmitter of *Mungbean yellow mosaic virus* were obtained from the glass house of Department of plant pathology, AC& RI, TNAU Coimbatore where it was mass cultured in a controlled environment. (Figure.1).

Green gram leaves

The MYMV susceptible greengram seeds CO – 8 variety were sprouted first and were sown in the pot with the potting mixture as redsoil : sand : Vermicompost @ 1:1:1 ratio. The 2nd trifoliate, leaves were collected for the experiment.

Preparation of nanoformulation

The azadirachtin was loaded in silica nanoparticle using a solvent evaporation technique. The 100mg of silica nanoparticle was dispersed in ethanol for 10 mins and 1 mg of Azadirachtin was added and stirred for 2 hr in dark condition. The silica-based nanoformulation of Azadirachtin, imidacloprid, Annosqumosin of 10ppm/mg, was dissolved in water to prepare different concentration such as 100ppm, 250ppm and 500ppm. Distilled water was used as control.

Bioassay technique

The adult whiteflies of, less than 10 days old were tested using a detached leaf assay technique. There are different bioassay methods for evaluation of the insecticidal activity. It includes topical application, film method, potters tower method, dipping method (larval and leaf dip), injection method and contact and residual method (Marimuthu, Subramanian, & Mohan, 1981).

In this study, injection method was used. The fresh second trifoliate leaves were taken in a Petri plate and 500 microlitres of 3 different nano formulation at each dosage was sprayed over the veins of the leaves (Figure: 2). They were kept undisturbed for an hour such that the water will evaporate. Then the leaves were tied using a thread and inserted in the assay container containing whiteflies and the mouth covered with muslin cloth for proper ventilation and incubated in ambient temperature (Figure: 3). The experiment was conducted as a factorial completely randomized design with time and treatments as a variable factor. The experiment was conducted with 13 treatments with 3 replications. Ten insects per replication were introduced. The 13 treatment includes Control,100ppm Azadirachtin loaded in nano silica, 250ppm Azadirachtin loaded in nano silica, 500ppm Azadirachtin loaded in nano silica, 100ppm imidacloprid loaded in nano silica, 250ppm imidacloprid loaded in nano silica, 500ppm imidacloprid loaded in nano silica, 100ppm of Annosquomisin, 250ppm Annosquomisin, 500ppm Annosquomisin, 100ppm Silica nano particles, 250ppm Silica nano particles, 500 Silica nano particle.

Statistical analysis

Bioassay data were pooled and LC 50 values were found using MS Excel program through probit analysis. All the data were subjected to two way ANOVA test in SPSS.16 software to find the significances of the treatments.

RESULTS AND DISCUSSION

The azadirachtin was loaded in silica nanoparticle with loading capacity and encapsulation efficiency of 4 percent and 12 percent respectively (Unpublished

Figure 1. *Bemisia tabaci* mass cultured on cotton plants

TABLE 1. Percent Mortality of Bemissia tabaci at different hours of application of treatments

Treatment/Time(hr)	1	3	5	7	9	11	13	15
Control (T1)	0	0	0	10	13.33	20	33.33	50
	(0)	(0)	(0)	(18.43)	(21.41)	(26.56)	(35.26)	(45)
100ppm Azadirachtin loaded in nano silica (T2)	16.66	26.66	40	66.66	73.33	86.66	90	96.66
	(24.09)	(31.09)	(39.23)	(54.73)	(58.90)	(68.58)	(71.56)	(79.48)
250ppm Azadirachtin loaded in nano silica (T3)	26.66	33.33	43.33	90	96.66	96.66	100	100
	(31.09)	(35.26)	(41.16)	(71.56)	(79.48)	(79.48)	(90)	(90)
500ppm Azadirachtin loaded in nano silica (T4)	46.66	76.66	96.66	100	100	100	100	100
	(43.08)	(61.11)	(79.48)	(90)	(90)	(90)	(90)	(90)
100ppm Annosquamosin (T5)	10	23.33	43.33	53.33	66.66	76.66	86.66	93.33
	(18.43)	(28.88)	(41.16)	(46.91)	(54.73)	(61.11)	(68.58)	(75.03)
250ppm Annosquamosin (T6)	30	33.33	46.66	70	86.66	90	100	100
	(33.21)	(35.26)	(43.08)	(56.78)	(68.58)	(71.56)	(90)	(90)
500ppm Annosquamosin (T7)	33.33	53.33	70	90	96.66	96.66	100	100
	(35.26)	(46.91)	(56.78)	(71.56)	(79.48)	(79.48)	(90)	(90)
100ppm Imidacloprid loaded in nano silica (T8)	40	53.33	60	66.66	80	83.33	90	96.66
	(39.23)	(46.91)	(50.76)	(54.73)	(63.43)	(65.90)	(71.56)	(79.48)
250ppm Imidacloprid loaded in nano silica (T9)	36.67	56.66	70	90	100	100	100	100
	(37.26)	(48.83)	(56.78)	(71.56)	(90)	(90)	(90)	(90)
500ppm Imidacloprid loaded in nano silica (T10)	36.67	53.33	83.33	100	100	100	100 (90)	100
	(37.26)	(46.91)	(65.90)	(90)	(90)	(90)		(90)
100ppm nano silica (T11)	33.33	43.33	60	70	83.33	76.66	93.33	100
	(35.26)	(41.16)	(50.76)	(56.78)	(65.90)	(71.56)	(75.03)	(90)
250ppm nano silica (T12)	30	40	50	63.33	73.33	96.66	93.33	100
	(33.21)	(39.23)	(45)	(52.73)	(58.90)	(61.15)	(75.03)	(90)
500ppm nano silica (T13)	23.33	40	63.33	90	96.66	96.66	100	100
	(28.88)	(39.23)	(52.73)	(71.56)	(79.48)	(79.48)	(90)	(90)
SED	4.402							
CD	8.678							

data). There was a significant difference in treatments with respect to time and concentration with the p-value less than 0.05. This was confirmed through 2 way ANOVA analysis. The mean value of mortality along with standard deviation is given in Table1. The susceptibility *Bemisiatabaci*, to a different

Figure 2. Detached leaves sprayed with nanoformulations; (A): 100ppm nano silica; (B): 250ppm nano silica; (C): 500ppm nano silica; (D): 100ppm of Azadirachtin loaded in nano silica; (E): 250ppm of Azadirachtin loaded in nano silica; (F): 500ppm of Azadirachtin loaded in nano silica; (G): 100ppm of Annosquamosin; (H): 250ppm of Annosquamosin; (I): 500ppm of Annosquamosin; (J): 100ppm of Imidacloprid loaded in nano silica; (K): 250ppm of Imidacloprid loaded in nano silica; (L): 500ppm of Imidacloprid loaded in nano silica

formulation at varying time interval was shown in the figure:5. The 500ppm Azadirachtin showed the cent per cent mortality percentage during 5thhr incubation. This was followed by 90% percent mortality due to 500ppm Imidacloprid, 500ppm Annosquamosin and 250ppm Azadirachtin formulations followed by 500ppm Annosquamosin and 250ppm Annosquamosin whereas in control at 5thhr of incubation the mortality rate was only 10%. Half dose of imidacloprid is sufficient for controlling the whiteflies.

Figure 3. Experimental setup containing insecticide treated leaves and *Bemisia tabaci*

In all the 4 sets of treatments, the percentage of mortality increased with increase in concentration and time. At 5th hour of incubation, the mortality per cent in Azadirachtin, imidacloprid and Annosquamosin were 66.66%, 53.33% and 66.66% at 100ppm, 90%, 70% and 80% at 250ppm and finally, 100%, 90% and 90% at 500ppm respectively.

Figure 4. Bemisia tabaci feeding insecticide treated leaves of green gram

The present results are in parallel with the results of (Pinheiro et al., 2009). As they reported that the mortality increases with increase in the concentration of neem oil. The commercial neem oil formulation of 0.5% and 1.0% showed mortality of 97% and 99% respectively.

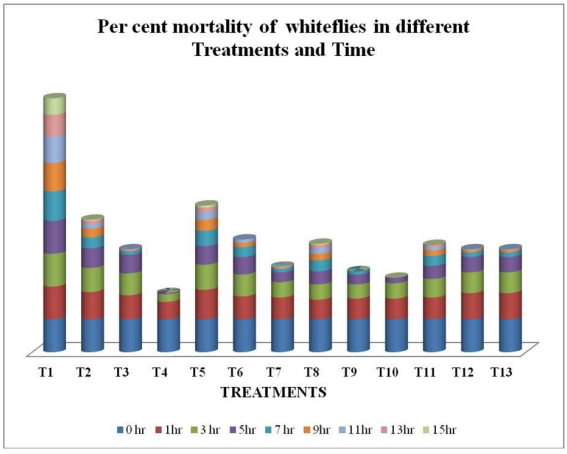

Figure 5. Percent mortality due to different treatments at varying time interval; T1: Control; T2:100ppm Azadirachtin loaded in nano silica; T3:250ppm Azadirachtin loaded in nano silica; T4 :500ppm Azadirachtin loaded in nano silica; T5: 100ppm imidacloprid loaded in nano silica; T6: 250ppm imidacloprid loaded in nano silica; T7: 500ppm imidacloprid loaded in nano silica; T8: 100ppm of Annosquomicin; T9: 250ppm Annosquomicin; T10: 500ppm Annosquomicin; T11: 100ppm Silica nano particles; T12: 250ppm Silica nano particles; T13: 500 Silica nano particle.

The mortality rate of *Helicoverpaarmigera*larva was increasing with the increasing concentration of *Annosquamosin*(Pareek et al., 2018). (Alim et al., 2017)reported the mortality of whiteflies after 48 hr of incubation with Annosquamosin.

Table 2. LC 50 and LC 90 values of different formulations

Treatments	LD50 (ppm)	LD90 (ppm)	P value
Silica nanoparticles loaded with Azadirachtin	87.09	363.07	0.001
Annosquamosin	48.97	3890.41	0.001
Nano silica	35.48	4.462	0.001

Apart from biomolecule, the carrier molecule by itself is showing an inhibitory effect on *Bemissia tabaci*. The mortality per cent due to nano silica at the 5th hour is 70% for 100ppm, 63.33% for 250ppm and 86.67% for 500ppm. It's almost equal to the inhibitory effect shown by imidacloprid and Annosquamosin. Interestingly, the mortality of whiteflies decreased at 250ppm and again increased at 500ppm. The fragile nature of the insects body and its stylet may be damaged due to the silica nanoparticles (Figure: 4). It may lag food for hours and then died due to starvation. Pavithra et al, 2018 reported that increasing the concentration of silica nanoparticles, the mortality of mealybugs and aphids were increased. The 3rd instar of S*podoptera literal* mouthparts and cuticles were damaged due to silica nanoparticles. The insects were died due to starvation, dehydration followed by desiccation (Debnath et al. (2011)).

The LC 50 and LC 90 values were found using probit analysis and are represented in Table2, The LC50 value of Azadirachtin (87.09 ppm) is higher than the annosquamosin (48.97 ppm) formulation, whereas LC 90 value of Azadirachtin (363.07 ppm) is lower than the Annosquamosin (3890.41 ppm). This is due to the controlled release of Azadirachtin from silica nanoparticles which prolonged the activity providing less concentration for higher mortality. Silica nanoparticle's LC 50 and LC 90 value are 35.48ppm and 4.07ppm which clearly indicates that for killing the larger population of whiteflies low dosage is required while for smaller population high dosage is required. This is in contrast with the reports of (Pavithra et al, 2018) that at higher concentration of nanosilica, more will be the mortality. From, the results it was obvious that increasing the concentration of silica reduces the mortality rate. At higher concentration due to the low stability of nanoparticle -19.6 mV (Unpublished data), the particles get agglomerated, thus comparatively large silica nanoparticles have reduced the effect on *Bemisia tabaci.* The LC 50 and LC 90 values of azadirachtin loaded silica nanoparticles was less than the silica nanoparticles, this may be due to the increased size of silica nanoparticle in azadirachtin loaded silica nanoparticle. It may also be due to the reduction of undulated surface, the major cause of death due to silica nanoparticle.

CONCLUSION

This study provides a better alternative to replace chemical pesticides. The LD 90 of Azadirachtin loaded silica nanoparticles was 363.07 ppm. Since the cent per cent mortality happened at 5 hrs, it may be a higher concentration for controlling whiteflies. Hence further investigation on the lower dosage which has high mortality in longer interval needs to be determined.

REFERENCES

Alim, M. A., Song, J., Lim, U.T., Choi, J.J., and Hossain, M.A. (2017). Bioassay of plant extracts against Aleurodicus dispersus (Hemiptera: Aleyrodidae). *Florida Entomologist*, 350-357.

Bag, D. (2000). Pesticides and health risks. *Economic and Political Weekly*, 3381-3383.

Bethke, J. A., Paine, T. D., and Nuessly, G.S. (1991). Comparative biology, morphometrics, and development of two populations of Bemisia tabaci (Homoptera: Aleyrodidae) on cotton and poinsettia. *Annals of the Entomological Society of America, **84**(4)*, 407-411.

Chaudhary, S., Kanwar, R. K., Sehgal, A., Cahill, D. M., Barrow, C. J., Sehgal, R., and Kanwar, J. R. (2017). Progress on Azadirachta indica based biopesticides in replacing synthetic toxic pesticides. *Frontiers in plant science, **8***, 610.

Chu, C. C., Henneberry, T. J., and Cohen, A. C. (1995). Bemesia argentifolii (Homoptera: Aleyrodidae): host preference and factors affecting oviposition and feeding site preference. *Environmental Entomology, **24**(2)*, 354-360.

Debnath, N., Das, S., Seth, D., Chandra, R., Bhattacharya, S. C., and Goswami, A. (2011). Entomotoxic effect of silica nanoparticles against Sitophilus oryzae (L.). *Journal of Pest Science, **84**(1)*, 99-105.

Fishpool, L., and Burban, C. (1994). Bemisia tabaci: the whitefly vector of African cassava mosaic geminivirus. *Tropical Science, **34**(1)*, 55-72.

Flores-Céspedes, F., Martínez-Domínguez, G. P., Villafranca-Sánchez, M., and Fernández-Pérez, M. (2015). Preparation and characterization of azadirachtin alginate-biosorbent based formulations: water release kinetics and photo degradation study. *Journal of agricultural and food chemistry, **63**(38)*, 8391-8398.

Jadhav, D.R., and Armes, N. J. (1996). Comparative status of insecticide resistance in the Helicoverpa and Heliothis species (Lepidoptera: Noctuidae) of south India. *Bulletin of entomological research, **86**(5)*, 525-531.

Lai, S., and Pillai, P. (1982). Ecological studies on whitefly. *Bemisia tabaci*, 101-102.

Lal, S. (1985). A review of insect pests of mungbean and their control in India. *International Journal of Pest Management, **31*(2), 105-114.

Marimuthu, T., Subramanian, C., and Mohan, R. (1981). Assessment of yield losses due to yellow mosaic infection in mungbean. *Pulse Crops Newsletter, 1*, 104.

Markham, P., Bedford, I., Liu, S., Frolich, D., Rosell, R., and Brown, J.K. (1996). The transmission of geminiviruses by biotypes of Bemisia tabaci (Gennadius) *Bemisia: 1995. Taxonomy, biology, damage, control and management* (pp. 69-75).

McAuslane, H. J. (2002). *Sweetpotato Whitefly B Biotype of Silverleaf Whitefly, Bemisia tabaci (Gennadius) or Bemisia argentifolii Bellows & Perring (Insecta: Homoptera: Aleyrodidae)*: University of Florida Cooperative Extension Service, Institute of Food and Misra, R. B. C. L., & Singh, K. (1929). *Cotton White-Fly.(Bemisia Gossypiperka, N. Sp.)*.

Naresh, J., and Nene, Y. (1980). Host range, host preference for oviposition and development and the dispersal of Bemisia tabaci Gennadius, a vector of several plant viruses. *Indian Journal of Agricultural Sciences, 50*(8), 620-623.

Nene, Y. (1972). A survey of the viral diseases of pulse crops in India. *Indian J. Res. Bull, 4*, 191.

Nene, Y. (1973). Viral diseases of some warm weather pulse crops in India. *Plant Disease Reporter, 57*(5), 463-467.

Pareek, C., Jadhav, M., Rokade, H., Jatale, A., and Pavitrakar, N. (2018). Laboratory evaluation of custard apple seed extract against Pigeon pea Pod Borer Helicoverpa armigera (Hubner). *Journal of Entomology and Zoology Studies, 6*(1), 541-543.

Pavela, R. (2014). Limitation of plant biopesticides *Advances in plant biopesticides* (pp. 347-359): Springer.

Pinheiro, P.V., Quintela, E.D., Oliveira, J.P.D., and Seraphin, J. C. (2009). Toxicity of neem oil to Bemisia tabaci biotype B nymphs reared on dry bean. *Pesquisa Agropécuaria Brasileira, 44*(4), 354-360.

Trehan, K., and Bagal, S. (1944). *Life-history and bionomics of potato tuber moth (Phthorimaea operculella Zell., Lepidoptera: Gelechiidae).* Paper presented at the Proceedings of the Indian Academy of Sciences-Section B.

Yadav, I. C., Devi, N. L., Syed, J. H., Cheng, Z., Li, J., Zhang, G., and Jones, K. C. (2015). Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India. *Science of the Total Environment, 511*, 123-137.