

# Phytochemical screening and the toxic effect of *Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* against white fly, *Bemisia tabaci*.

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## ABSTRACT

*The present study was designed to evaluate the toxicity of different botanicals against the whitefly Bemisia tabaci. Methanolic and aqueous extracts of different plants (Azadirachta indica, Zingiber officianalis and Annona squamosa) known for their medicinal value were prepared by Soxhlet extraction which were then evaluated for insecticidal activity against eggs and nymphs of Bemisia Tabaci at different concentrations. The results showed that the methanolic and aqueous extracts of Azadirachta indica showed high to moderate insecticidal activity against eggs and nymphs (79.00% and 100.00±0.1) followed by Zingiber officianalis (76.09% and 99.12± 0.1) at 25 % concentrations after 72 hours of treatment respectively. The aqueous extract of Annona squamosa showed least toxic effect (56.29 % and 50.00) at 5% concentration after 48 hours of*

*treatment. A gradual increase in the mortality was found with the increase of concentration and duration of the treatment. The overall efficacy of the three pesticides against the survivorship was in the order of Azadirachta indica > Zingiber officianalis > Annona squamosa. Hence, the extracts from these plants could be used as an effective and environmentally sustainable bio-insecticide for the control of whiteflies, Bemisia tabaci.*

**Keywords:** Bemisia Tabaci, Mortality, Botanical Extracts, Azadirachta indica, Zingiber officianalis, Annona squamosa

## INTRODUCTION

Cotton (*Gossypium hirsutum L.*), is the King of fibre popularly known as White gold, an important cash crop in India. Amongst various causes of low yield, losses due to insect-pests are one of the important factors. About 200 insect pests are reported to attack

cotton crop in India (**Anonymous, 1992**). Many insect-pests are encountered on cotton crop from germination to harvesting. Out of them whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is widely distributed polyphagous pest in tropical and sub tropical regions of India. Cotton is high value fiber crop that lead to heavy loss of economy due to permanent injury caused by whitefly. maximum populations of *B. tabaci* cause losses in plant productivity by fungal growth, direct feeding, plant physiological disorders and associated with honey dew contamination. Losses also occur from *B. tabaci* due to the efficient transmission of *Begomo virus*, a genus of the taxonomic family *Geminiviridae* that causes leaf yellow mosaic and mottling, leaf distortion and stunting (**Oliveira et al., 2001; Morales, 2007**). The management of *B. tabaci* has been typically carried out by chemical insecticides, which are associated with environmental contamination, high levels of resistance and damage to non-target organisms (**Elberth and Nauen, 2000; Reditakis et al., 2009**). Currently, plant based pesticides have been considered one of the most promising sources of bio-rational products with new modes of actions to manage phytophagous insects, (**Dayan et**

**al., 2009; Rattan 2010**). . Plants provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Insecticidal activity of many plants against several insect pests has been demonstrated. The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, anti-feedant growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility (**Jbilou et al., 2006**) Based on the above mentioned importance of the pest there is a dire need to manage this insect in a way that is environmentally friendly and economically feasible. The present study was therefore carried out to evaluate the efficacy of botanical extracts *Azadirachta indica*, *zingerber officianalis* and *Annona squamosa* for the control of cotton whitefly

## **MATERIALS AND METHODS**

### **Experimental plants used for treatment:**

Following plants were selected to obtain their extracts-

1. *Azadirachta indica* (leaves)
2. *Annona Squamosa* (leaves)
3. *Zingiber officinales* (roots)

### **Collection of medicinal plant:**

Selected plant materials i.e. leaves of *Azadirachta indica* and *Annona squamosa* were collected from in and around the Indore region in poly bags and brought to lab and their botanical identity was established and *Zingiber officinales* was brought from the market and was also identified at the department of botany, PMB Gujarati science college, Indore (M.P.). The collected material was washed, shade dried under room temperature ( $27 \pm 2^{\circ}$  C) and *Azadirachta indica* and *Annona squamosa* was powdered using electric blender and the paste of *Zingiber officinales* was made which were further processed for phytochemical analysis.

### **Soxhlet extraction: (Sharma and Gupta, 2009)**

The ordinary method of extraction was not efficient to yield good amount of active principle of the plant material. So for that reason Soxhlet extraction was done.

### **Phytochemical analysis**

Chemical tests were undertaken to identify the phytochemicals namely alkaloids, flavonoids, saponins, terpenoids, tannins,

glycosides, steroids as per methodology adopted by **Harborne (1998)**.

### **Collections and rearing of insects (Muniz and Nombela 2001)**

Adult whiteflies were collected from the cotton fields. The stock of colony of *Bemesia tabaci* was maintained on cotton plants in entomological cages. The cages were then kept in greenhouse at 25-35°C, 55-75% relative humidity and natural light. Bioassays were then carried out on *Bemesia tabaci* eggs and nymphs. Groups of 15 *Bemesia tabaci* adults were deposited into cages (2cm dia.) set on cotton leaves of 25-30 days old plants. After an ovipositional period of 48 hours, all adult flies were removed and plants were kept in cages for 72 hours for egg bioassays and 15 days for nymph bioassays.

### **Toxicity Assays**

Toxicity tests were carried out by the method given by **(Angel et al., 2013)**. Then the percentages of corrected mortality were calculated by using Abbott's formula **(Abbott, 1925)**.

$$Mc = (Mo - Mc / 100 - Me) \times 100$$

Where, Mo = Observed mortality rate of treated larvae (%),

Me = Mortality rate of control (%)

Mc = Corrected mortality rate (%)

## RESULTS

### PHYTOCHEMICAL SCREENING:

The phytochemical screening of *Azadirachta indica* leaves showed the presence of phenolics, flavonoid, saponins, glycosides, steroids, tannins and alkaloids in both methanolic and aqueous extract.

The phytochemical screening of *zingiber officianalis* (root) showed the presence of phenolics, flavonoid, saponins, glycosides

and alkaloids and absence of steroids and tannins aqueous extract and presence of phenolics, flavonoid, steroids, tannins, glycosides and alkaloids and absence of saponins.

The phytochemical screening of *Annona squamosa* (leaves) showed the presence of phenolics, flavonoid, tannins and absence of saponins, glycosides, alkaloids and steroids in aqueous extract and presence of flavonoid, tannins and alkaloids and absence of phenolics, steroids, saponins and glycosides.

**Table-1 Phytochemical screening of *Azadirachta indica*, *Zingiber Offcinale* and *Annona Squamosa*.**

| PLANTS USED               | PHYTOCHEMICALS | METHANOLIC EXTRACT | AQUEOUS EXTRACT |
|---------------------------|----------------|--------------------|-----------------|
| <i>Azadirachta indica</i> | Glycosides     | +                  | +               |
|                           | Steroids       | +                  | +               |
|                           | Phenolics      | +                  | +               |
|                           | Flavonoids     | +                  | +               |
|                           | Tannins        | +                  | +               |
|                           | Alkaloids      | +                  | +               |
|                           | Saponins       | +                  | +               |
| <i>Zingiber Offcinale</i> | Glycosides     | +                  | +               |
|                           | Steroids       | +                  | —               |
|                           | Phenolics      | +                  | +               |
|                           | Flavonoids     | +                  | +               |
|                           | Tannins        | +                  | —               |
|                           | Alkaloids      | +                  | +               |

|                        |            |   |   |
|------------------------|------------|---|---|
|                        | Saponins   | - | + |
| <i>Annona Squamosa</i> | Glycosides | - | - |
|                        | Steroids   | - | - |
|                        | Phenolics  | - | + |
|                        | Flavonoids | + | + |
|                        | Tannins    | + | + |
|                        | Alkaloids  | + | - |
|                        | Saponins   | - | - |

### Toxicity Bioassay

In the present study toxicity tests of *Bemisia tabaci* eggs and nymphs were carried in methanolic and aqueous extracts of three different plants *Azadirachta indica*, *Zingiber Officinale* and *Annona Squamosa* at different concentrations ranging from 5-25% are summarized in table 2-6 and presented by figure 1-5.

The maximum percent mortality of white fly (*bemisia tabaci*) at 72 hours of duration was showed by methanolic extracts of *Azadirachta indica*-leaves ( $100.0 \pm 0.0\%$ ) followed by *Zingiber officianalis* ( $99.12 \pm 0.1$ ) at 25 % concentration and the minimum percent mortality was showed by aqueous extract of *Annona squamosa* i.e.  $65.01 \pm 0.2$  at 5.0% concentration

The maximum percent mortality of white fly (*bemisia tabaci*) nymph at 48 hours of duration was showed by methanolic

extracts of *Azadirachta indica*-leaves ( $96.66 \pm 0.1\%$ ) followed by *Zingiber officianalis* ( $95.87 \pm 0.2\%$ ) at 25 % concentration and the minimum percent nymph mortality was showed by aqueous extract of *Annona squamosa* i.e.  $64.91 \pm 0.3$  at 5.0% concentration .

The maximum percent mean of unhatched eggs of white fly (*bemisia tabaci*) at 72 hours of duration was showed by methanolic extracts of *Azadirachta indica*-leaves (79.00%) followed by *Zingiber officianalis* (76.09%) at 25 % concentration and the minimum percent mortality was showed by aqueous extract of *Annona squamosa* i.e. 56.29 % at 5.0% concentration

The maximum percent mean of unhatched eggs of white fly (*bemisia tabaci*) at 72 hours of duration was showed by methanolic extracts of *Azadirachta indica*-

leaves (66.75%) followed by *Zingiber officianalis* (65.97%) at 25 % concentration and the minimum percent mortality was showed by aqueous extract of *Annona squamosa* i.e. 50.00 % at 5.0% concentration

Data pertaining to the below tables indicate that the Methanolic extract of *A. indica* showed the maximum ( $100.0 \pm 0.0\%$ ) percentage of nymph mortality followed by aqueous extract of *A. indica* both at 25 % concentration and than *Z. officianales* ( $99.12 \pm 0.1$ ) at 25 % concentration after 72

hours of treatment and lowest percentage of nymph mortality was showed by aqueous extract *A. squamosa* at 5.0 % concentration after 48 hours of duration. The result also indicated the mean of unhatched eggs of white fly, maximum mean of unhatched eggs were showed by *Azadirachta indica* (79.00 %) followed by *Zingerber officianalis* (76.09) at 25 % concentration after 72 hours of treatment and the least number of unhatched eggs were shown by the aqueous extract of *Annona squamosa* (50.00) at 5.0 % of concentration after 48 hours of treatment.

**Thus the above result reveals that as the concentration of plant extracts increases, percentage of nymph mortality and unhatched eggs also increases. Therefore in general, the toxic effects of different concentrations, irrespective of the extracts decrease with decrease in concentration from 5.0% to 25 %.**

**Table-2: Mortality of *Bemesia tabaci* nymph by methanolic and aqueous extracts of**

| Name of the plants           | Concentration (%) | % mortality of white fly Nymphs     |                                  |
|------------------------------|-------------------|-------------------------------------|----------------------------------|
|                              |                   | Methanolic extract (Mean $\pm$ SEM) | Aqueous extract (Mean $\pm$ SEM) |
| <i>Azadirachta indica</i>    | 5                 | 73.19 $\pm$ 0.1                     | 73.06 $\pm$ 0.2                  |
|                              | 10                | 92.37 $\pm$ 0.3                     | 92.01 $\pm$ 0.5                  |
|                              | 15                | 94.16 $\pm$ 0.4                     | 94.00 $\pm$ 0.1                  |
|                              | 20                | 95.11 $\pm$ 0.2                     | 94.28 $\pm$ 0.0                  |
|                              | 25                | 96.66 $\pm$ 0.1                     | 96.40 $\pm$ 0.3                  |
|                              | Control           | 15.32 $\pm$ 0.0                     | 15.32 $\pm$ 0.0                  |
| <i>Zingiber officianalis</i> | 5                 | 72.70 $\pm$ 0.2                     | 69.00 $\pm$ 0.1                  |
|                              | 10                | 89.61 $\pm$ 0.5                     | 86.22 $\pm$ 0.3                  |
|                              | 15                | 94.15 $\pm$ 0.0                     | 94.00 $\pm$ 0.2                  |
|                              | 20                | 95.11 $\pm$ 0.3                     | 94.17 $\pm$ 0.5                  |
|                              | 25                | 95.87 $\pm$ 0.2                     | 95.33 $\pm$ 0.1                  |
|                              | Control           | 15.32 $\pm$ 0.0                     | 15.32 $\pm$ 0.0                  |
| <i>Annona squamosa</i>       | 5                 | 66.06 $\pm$ 0.0                     | 64.91 $\pm$ 0.3                  |
|                              | 10                | 74.13 $\pm$ 0.1                     | 69.02 $\pm$ 0.5                  |
|                              | 15                | 81.60 $\pm$ 0.3                     | 76.35 $\pm$ 0.1                  |
|                              | 20                | 84.05 $\pm$ 0.1                     | 81.59 $\pm$ 0.2                  |
|                              | 25                | 89.18 $\pm$ 0.2                     | 88.79 $\pm$ 0.3                  |
|                              | Control           | 15.32 $\pm$ 0.0                     | 15.32 $\pm$ 0.0                  |

*Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* in different concentrations at 48 hours after treatment.

Mean mortality ( $\pm$  standard error),  $p < 0.05$ .

**Table-3: Mortality of *Bemisia tabaci* nymphs by methanolic and aqueous extracts of *Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* in different concentrations at 72 hours after treatment.**

| Name of the plants           | Concentration (%) | % mortality of white fly Nymphs     |                                  |
|------------------------------|-------------------|-------------------------------------|----------------------------------|
|                              |                   | Methanolic extract (Mean $\pm$ SEM) | Aqueous extract (Mean $\pm$ SEM) |
| <i>Azadirachta indica</i>    | 5                 | 75.01 $\pm$ 0.2                     | 73.50 $\pm$ 0.3                  |
|                              | 10                | 94.33 $\pm$ 0.1                     | 94.00 $\pm$ 0.1                  |
|                              | 15                | 95.21 $\pm$ 0.3                     | 94.76 $\pm$ 0.2                  |
|                              | 20                | 98.65 $\pm$ 0.0                     | 97.92 $\pm$ 0.5                  |
|                              | 25                | 100 $\pm$ 0.2                       | 99.30 $\pm$ 0.0                  |
|                              | Control           | 15.32 $\pm$ 0.0                     | 15.32 $\pm$ 0.0                  |
| <i>Zingiber officianalis</i> | 5                 | 73.00 $\pm$ 0.1                     | 69.03 $\pm$ 0.3                  |
|                              | 10                | 90.08 $\pm$ 0.5                     | 86.28 $\pm$ 0.1                  |
|                              | 15                | 95.16 $\pm$ 0.2                     | 94.23 $\pm$ 0.3                  |
|                              | 20                | 98.00 $\pm$ 0.4                     | 97.06 $\pm$ 0.0                  |
|                              | 25                | 99.12 $\pm$ 0.1                     | 98.84 $\pm$ 0.2                  |
|                              | Control           | 15.32 $\pm$ 0.0                     | 15.32 $\pm$ 0.0                  |
| <i>Annona squamosa</i>       | 5                 | 67.38 $\pm$ 0.1                     | 65.01 $\pm$ 0.2                  |
|                              | 10                | 76.06 $\pm$ 0.0                     | 69.98 $\pm$ 0.0                  |
|                              | 15                | 82.37 $\pm$ 0.3                     | 76.88 $\pm$ 0.4                  |
|                              | 20                | 84.16 $\pm$ 0.2                     | 82.11 $\pm$ 0.3                  |
|                              | 25                | 89.80 $\pm$ 0.5                     | 89.00 $\pm$ 0.1                  |
|                              | Control           | 15.32 $\pm$ 0.0                     | 15.32 $\pm$ 0.0                  |

Mean mortality ( $\pm$  standard error),  $p < 0.05$ .



**Table-4: Percent Mean of unhatched *Bemesia tabaci* eggs by methanolic and aqueous extracts of *Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* in different concentrations at 48 hours after treatment.**

| Name of the plants           | Concentration (%) | %mean of unhatched white fly eggs |                        |
|------------------------------|-------------------|-----------------------------------|------------------------|
|                              |                   | Methanolic extract (Mean)         | Aqueous extract (Mean) |
| <i>Azadirachta indica</i>    | 5                 | 59.71                             | 59.11                  |
|                              | 10                | 61.24                             | 60.78                  |
|                              | 15                | 65.00                             | 64.55                  |
|                              | 20                | 65.10                             | 65.00                  |
|                              | 25                | 66.75                             | 66.17                  |
|                              | Control           | 12.97                             | 12.97                  |
| <i>Zingiber officianalis</i> | 5                 | 59.11                             | 59.08                  |
|                              | 10                | 60.58                             | 60.00                  |
|                              | 15                | 64.09                             | 62.76                  |
|                              | 20                | 64.83                             | 64.45                  |
|                              | 25                | 65.97                             | 65.01                  |
|                              | Control           | 12.97                             | 12.97                  |
| <i>Annona squamosa</i>       | 5                 | 50.31                             | 50.00                  |
|                              | 10                | 50.55                             | 50.28                  |
|                              | 15                | 54.43                             | 54.15                  |
|                              | 20                | 58.11                             | 56.00                  |
|                              | 25                | 60.97                             | 60.06                  |
|                              | Control           | 12.97                             | 12.97                  |

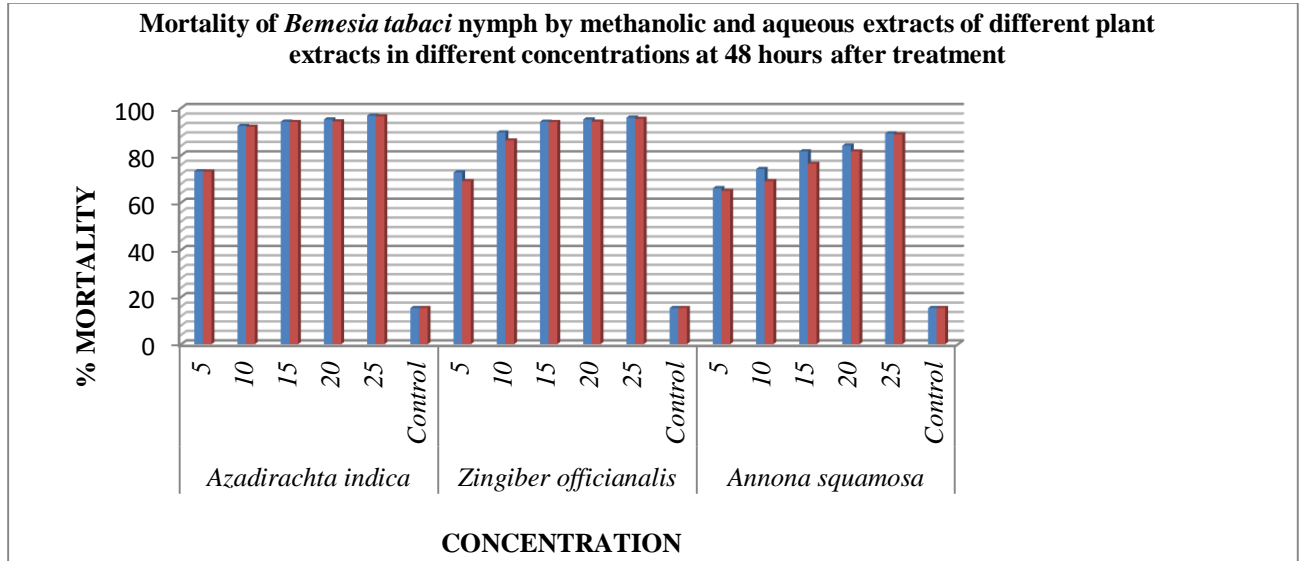
Mean mortality ( $\pm$  standard error),  $p < 0.05$ .

**Table-5: Percent Mean of unhatched *Bemesia tabaci* eggs by methanolic and aqueous extracts of *Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* in different concentrations at 72 hours after treatment.**

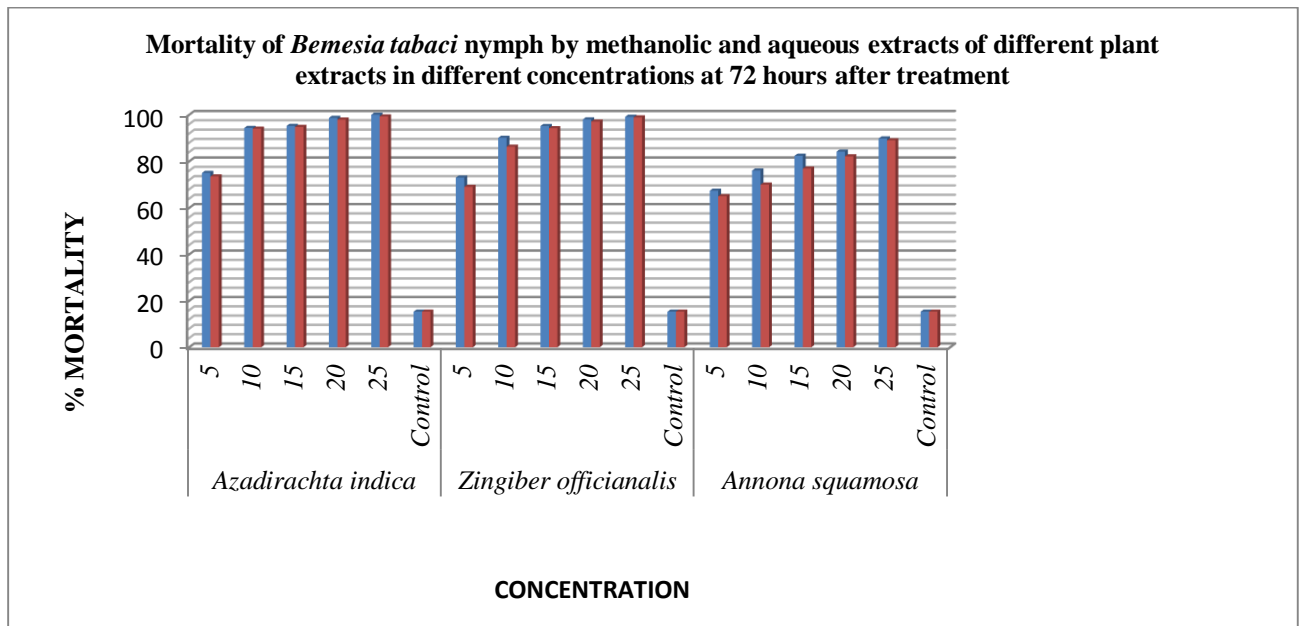
| Name of the plants           | Concentration (%) | %mean of unhatched white fly eggs |                        |
|------------------------------|-------------------|-----------------------------------|------------------------|
|                              |                   | Methanolic extract (Mean)         | Aqueous extract (Mean) |
| <i>Azadirachta indica</i>    | 5                 | 60.33                             | 60.11                  |
|                              | 10                | 66.38                             | 65.87                  |
|                              | 15                | 70.12                             | 68.65                  |
|                              | 20                | 73.66                             | 70.91                  |
|                              | 25                | 79.00                             | 76.43                  |
|                              | Control           | 12.97                             | 12.97                  |
| <i>Zingiber officianalis</i> | 5                 | 60.09                             | 60.00                  |
|                              | 10                | 65.98                             | 64.17                  |
|                              | 15                | 68.55                             | 66.66                  |
|                              | 20                | 73.14                             | 71.45                  |
|                              | 25                | 76.09                             | 76.00                  |
|                              | Control           | 12.97                             | 12.97                  |
| <i>Annona squamosa</i>       | 5                 | 56.31                             | 56.29                  |
|                              | 10                | 56.04                             | 55.00                  |
|                              | 15                | 59.43                             | 55.17                  |
|                              | 20                | 60.88                             | 60.05                  |
|                              | 25                | 72.50                             | 71.67                  |
|                              | Control           | 12.97                             | 12.97                  |

Mean mortality ( $\pm$  standard error),  $p < 0.05$ .

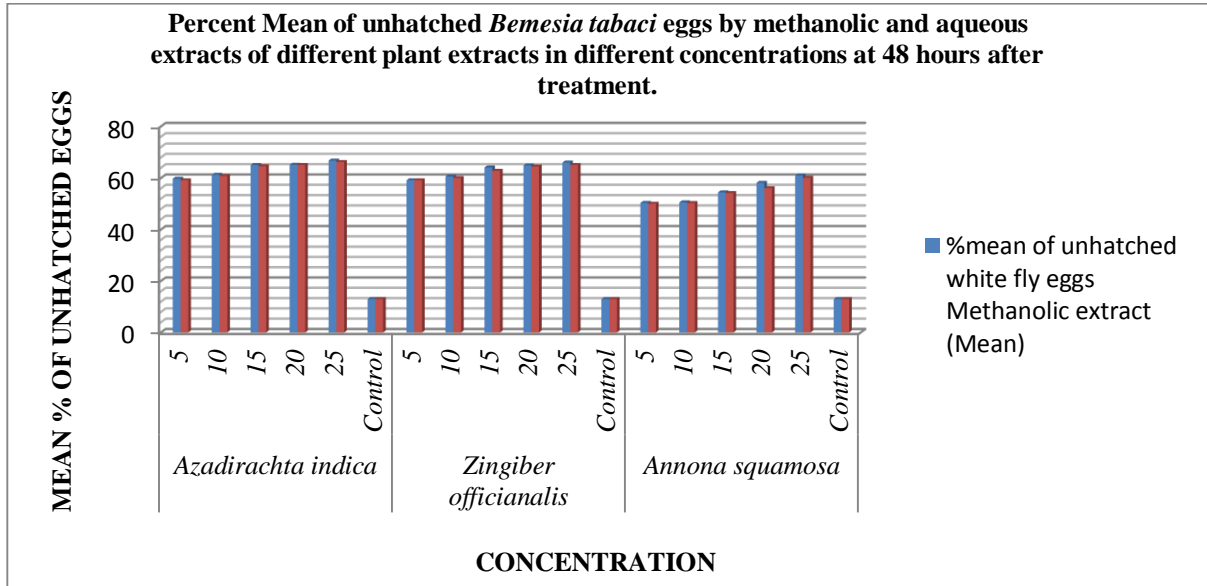
**Figure-1: Mortality of *Bemesia tabaci* nymphs by methanolic and aqueous extracts of *Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* in different concentrations at 48 hours after treatment.**



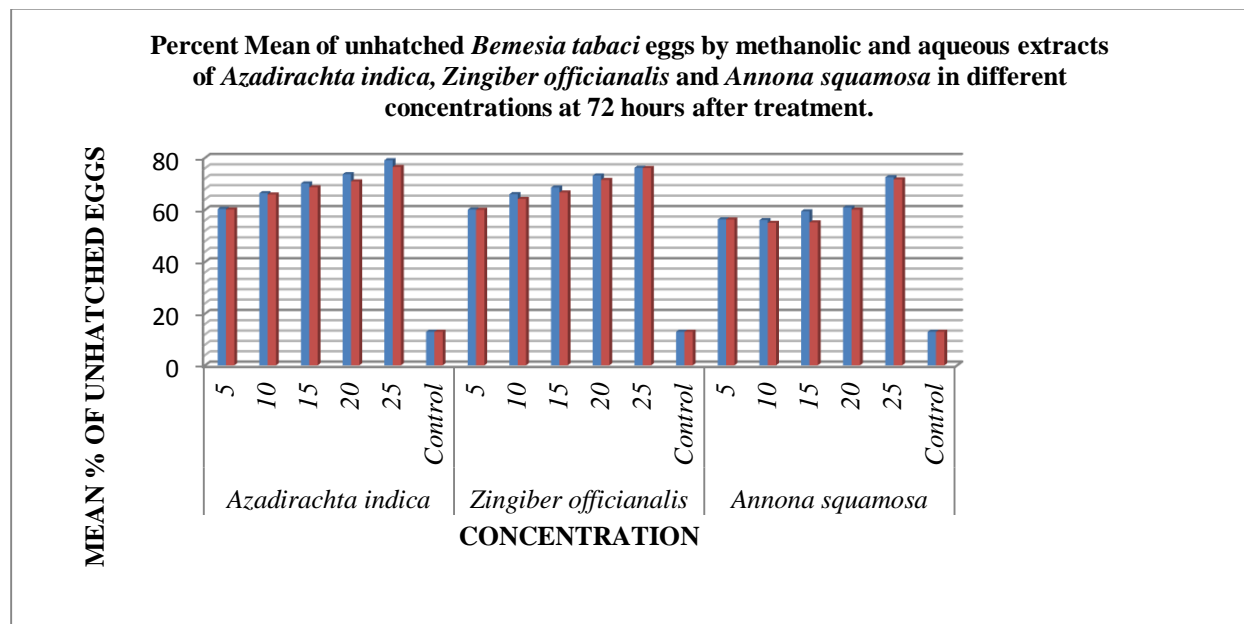
**Figure-2: Mortality of *Bemesia tabaci* nymphs by methanolic and aqueous extracts of *Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* in different concentrations at 72 hours after treatment.**



**Figure-3: Percent Mean of unhatched *Bemesia tabaci* eggs by methanolic and aqueous extracts of *Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* in different concentrations at 48 hours after treatment.**



**Figure-:- Percent Mean of unhatched *Bemesia tabaci* eggs by methanolic and aqueous extracts of *Azadirachta indica*, *Zingiber officianalis* and *Annona squamosa* in different concentrations at 72 hours after treatment.**



## DISCUSSION

Data pertaining to the below tables indicate that the Methanolic extract of *A. indica* showed the maximum ( $100.0 \pm 0.0\%$ ) percentage of nymph mortality followed by aqueous extract of *A. indica* both at 25 % concentration and than *Z. officianales* ( $99.12 \pm 0.1$ ) at 25 % concentration after 72 hours of treatment and lowest percentage of nymph mortality was showed by aqueous extract *A. squamosa* at 5.0 % concentration after 48 hours of duration. The result also indicated the mean of unhatched eggs of white fly, maximum mean of unhatched eggs were showed by *Azadirachta indica* (79.00 %) followed by *Zingerber officianalis* (76.09) at 25 % concentration after 72 hours of treatment and the least number of unhatched eggs were shown by the aqueous extract of *Annona squamosa* (50.00) at 5.0 % of concentration after 48 hours of treatment.

**Thus the above result reveals that as the concentration of plant extracts increases, percentage of nymph mortality and unhatched eggs also increases. Therefore in general, the toxic effects of different concentrations, irrespective of the**

**extracts decrease with decrease in concentration from 5.0% to 25 %.**

The results of present study the phytochemical screening indicated the presence of phenolics, alkaloids, flavonoids, steroids, glycosides, saponins, and tannins. The result is in agreement with the work of **Lawal et al. (2010)** who reported that garlic extract revealed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, cardiac glycosides, volatile oils and steroids. The result of present study of phytochemical analysis of *Azadirachta indica* (leaves) is also in agreement with **Kraus et al. (1981)**. Phytochemical analysis of the leaf extract of *Azadirachta indica* was reported by **Imran et al. (2010)** in which petroleum ether, chloroform and methanolic extracts was found to contain only glycosides, triterpenes and fatty acids in relatively higher quantities. Thus the above mentioned authors are in agreement with the present investigation.

Present study was also supported by **Garcia et al. (2007)** who also reported that aqueous, methanolic and dicloromethane extracts of leaves caused 100% mortality on the

whitefly. The study on pesticidal activity against *Sitophilus oryzae* revealed that, *Azadirachta* has shown significant activity when compared to other plants. The high performances of ethanolic extracts of *Azadirachta indica* in this study confirm the views of **Badam *et al.* (1987)**; **Jeyasakthy *et al.* (2013)** and **Udeinya *et al.* (2008)** that over 195 species of insects in West Africa, India, Myanmar etc are affected by aqueous and ethanolic neem extracts, and insects that have become resistant to synthetic pesticides are also controlled with these extracts.

**Abou *et al.* (2010)** tested the toxicity of *Ammimajus* (lace flower) and *Z. officinale* (ginger) either as formulation or as crude extracts against the nymphs of whiteflies and indicated that whiteflies can be effectively controlled with the application of the two extracts and their formulations

**Audrey (2004)** also found that crude seed extract of *A. squamosa* is a promising candidate as a botanical insecticide. The seeds of *A. squamosa* were reported to have insecticidal properties. At 2.5 percent concentrations, the crude oils from *A. squamosa* seeds significantly lowered the leaf injury caused by larvae (**Babu *et al.*,**

**1998**). **Kumar *et al.* (2010)** found that the extract of *Annona squamosa* being a contact poison for insects can penetrate the body wall and tracheal system bringing about death probably lending the extract the insecticidal activity against *Sitophilus oryzae*.

The present report suggest that plant extracts of *Azadirachta indica*, *Zingerber officinales* and *Annona squamosa* contain various compound that are biologically active, possesses a potentially vital toxic effect on *B. tabaci*. Thus, these plant extracts offers significant promise for combating the threat posed by white fly to farmers. The major thrust of this work is its adaptability for use by small scale farmers plagued by the challenge of not being able to afford conventional pesticides on the market.

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