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Comparative assessment of repellency effect of *Azadirachta indica*, *Zingiber officinale* and *Annona squamosa* extracts against cotton whitefly- *Bemisia tabaci*

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Abstract: **Cotton whitefly, *Bemisia tabaci* Gennadius is an invasive pest that infests many agricultural/food crops including cotton thereby reducing their productivity, quality and economic value. Due to the emerging pesticide resistance and its secondary side effects to the farmer community, there is a dire need to develop some safer and effective pest management strategies. With this rationale, we evaluated the comparative repellant potential of methanolic and aqueous extracts of three medicinal plants *Azadirachta indica, Zingiber officinale and Annona Squamosa* against cotton whitefly *Bemesia tabaci* (Gennadius) (Hemiptera: Aleyrodidae); using area preference method on filter papers discs under controlled conditions. The adult flies were exposed to varying concentrations of respective extracts ranging from 5-25% for 2, 4, 6, and 24 hours of duration. The results showed that methanolic extract of *Azadirachta indica* elicited the highest percent repellency (92.33%±11.42) followed by *Zingiber officinale* (90.05±10.5) at 25% concentration after 24 hours of treatment while as aqueous extract of *Annona Squamosa* showed the lowest percent repellency (5.34%±3.12) at 5% concentration after 2 hours of treatment . All of these plants were found significantly effective in repelling whitefly *Bemesia tabaci* and the repellency effect was found to significantly increase with increase in treatment time.**

Taken together the study describes the repellency potential of three medicinal plants against cotton whitefly *Bemesia tabaci*; however the active constituents need to be isolated and characterized to take the studies further towards translational setups in the field.

Keywords: **Cotton plant, *Bemisia tabaci*, whitefly, repellency, *Azadirachta indica, Zingiber officinale, Annona squamosal.***

1. INTRODUCTION

The cotton whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), a tiny white colored soft bodied sap-sucking plant pest is ranked one among the top 100 invasive organisms of the world [1]. It is one of the most noxious, economically injurious with a broadly polyphagous nature and is reported to infesting about 600 plant species [2]. The pest infests cotton plants decreasing their productivity; either directly feeding on phloem sap or due to fungal growth associated with honeydew (pest excreta) contamination which infact is the main cause of 'sticky cotton fibers' reducing its quality as well as marketability in many cotton producing countries [3, 4]. Additionally, *Bemesia tabaci* also transmits cotton leaf curl virus disease (CLCuV), a real threat to cotton production in India [5]. Its wider host adaptability, cryptic species nature and virus transmission potential has rendered its management even more difficult [6]. Consequently, till date the pest has developed resistance to more than 40 different ingredients of pesticides [7].

Insecticides have been the main source of weapon against *Bemisia tabaci* infestation in various agricultural systems. Key pests of cotton are controlled by broad-spectrum insecticides used in large quantity which cause not only health problems and environmental pollution but have also become the main reason of development of insecticide resistance against these insects [8, 9]. Consequently, the problems caused by synthetic insecticides on the environment and non-target organisms [10, 11] has stimulated the use of natural products as an alternative pest control strategy [12, 13]. These natural products have a lower persistence in the environment and, therefore are considered environmentally and toxicologically safer compared to several of the currently used synthetic pesticides. In addition botanical insecticides have repellent effects, inhibit oviposition and change the feeding and hormonal systems of several arthropod pests [12, 14]. Furthermore some essential oils and crude extracts of many plants have been well reported for repellant and insecticidal activity against potato sweet fly (*Bemesia tabaci*) [15].

Keeping in view the deleterious side effects posed by synthetic insecticides and the need to develop safer and effective insecticides of natural origin, we attempted to evaluate the pest repellency potential of *Azadirachta indica*, *Zingiber officinale* and *Annona squamosa* crude extracts on whitefly *Bemesia tabaci* under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Collections and rearing of insects

Adult whiteflies were collected from the cotton field. The stock of colony of *Bemesia tabaci* was maintained on cotton plants in entomological cages (1.2 x 1.2 x 1.0 m) under controlled conditions. The cages were kept in greenhouse at 25-35ºC, 55-75% relative humidity and natural light (12:12h).

2.2 Plant collection and extract preparation

The plant material- leaves of *Azadirachta indica* and *Annona squamosa* were collected from different places of Indore region in poly bags and *Zingiber officinale* was brought from local market. The respective plant specimens were identified and authenticated at Centre for Biodiversity and Taxonomy, University of Kashmir under voucher no. 2248-50/KASH herb dated 2016. The plants were shade dried, ground to powder and subjected to extraction in a Soxhlet extraction unit, using water or methanol as extraction solvents. The extraction was done for 2-3 days at 30-45°C and finally the extracts were filtered through whatman filter paper 1to remove suspended particulate. The filtrate was also spun at 500-700 rpm for 5 minutes to sediment small un-dissolved impurities and later subjected to dryness using a vacuum evaporator. The dry paste was stored in small vials at -80°C until further use.

2.3 Repellency bioassay

The repellant effects of the plant extracts were assessed by area preference method adopted as per the previously reported protocol with slight modifications [16]. Briefly, 90mm filter paper discs (Whattman filter 1) were divided and cut into two halves each and loaded either with the specified extract concentration or the corresponding extraction solvent (distilled water/ methanol) using a micropipette (200µL). Both the treated and the corresponding control filter paper halves were air dried; put one in each half of sterile petri-dishes (tarsons; cat no# 460092) attached using adhesive tape. The fifteen whitefly adults were placed at the center of each Petri dish and then covered with porous mesh cloth. Four replications of each treatment were maintained. The repellent effect of each plant extract was recorded by counting the number of adults present on the treated (T) (extract treated) and the control (C) (distilled water/methanol) strips after 2, 4, 6 and 24 hours. Percentage repellency (PR) values were computed using the formula below:

$$\% \text{ Repellancy} = \frac{C-T}{C+T} \times 100$$

Where C is the number of adult flies in control and T is the corresponding number in treated groups.

2.4 Statistical analysis

All statistical analyses and p value calculations were done using graph pad prism version 8.1.0 software. The p values were obtained by performing by performing one way ANNOVA by comparing average repellency values between varying treatment times as shown in respective figures. p values lesser than 0.05 were considered statistically significant at *p≤ 0.05, **p≤0.01, ***p ≤0.001. Results are expressed as average± standard deviation of four replicates.

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3. RESULTS

The pest repellant effects of methanolic and aqueous extracts were evaluated against white fly *(Bemesia tabaci)* in sterile petri dishes containing uniformly sized filter paper discs loaded with either solvent (control) or different concentrations of the extracts (5-25%). The individual percent repellency/ repellence index of each concentration for each individual replicate was calculated as shown in **table 1a-c & 2a-c**. The average repellence index of each concentration was also calculated and the results expressed as **mean ± S.D.** of four replicates as shown in **figure 1a-b**. From the results, it is clear that methanolic extracts showed higher repellent effects than corresponding aqueous extracts, though the variation was not too high. Further, all the extracts showed the effect/s in a concentration/ time dependent manner (Table 1a-c, 2a-c & Fig. 1a-b). Further, highest percent repellency was shown by methanolic/ aqueous extracts of *Azadirachta indica* (92.33%±11.42/ 88.53%±8.23) followed by *Zingiber officinale* (90.05%±10.5/ 83.27%±7.23) and *Annona squamosa* (88.93%±8.78/ 79.88%±8.76) at 25% concentration (24 hr. post treatment) while as at 5% concentration the respective pest repellencies were 67.80%±7.23/ 67.19%±6.45; 64.23%±6.34/ 58.23%±4.78 and 57.55%±7.45/ 43.89%±5.97. The highest percent repellency at 25% concentration after 2 hours of treatment was shown by methanolic/ aqueous extracts of *Azadirachta indica* (56.12%±8.12/ 52.35%±5.21) followed by *Zingiber officinale* (50%±6.41/ 47.16%±6.23) and *Annona squamosa* (42.18%±4.23/ 40.69%±4.65) while as the corresponding values at 5% concentration were 25.88%±4.23/ 21.35%±4.21, 18.03% ±3.5/ 13.44%±4.12 and 7.22%±3.54/ 5.34%±3.12.

The experiments were reproduced twice and the results were taken as the averages of the replicates.

Table 1: Repellency index of Methanolic extracts of **a)** *Azadirachta indica* **b)** *Zingiber officinale* and **c)** *Annona squamosa* extract obtained on different exposure times (2-24 hr. post treatment) at indicated concentrations in a filter paper bioassay against whitefly *Bemesia tabaci*

Table 1a):

2 hr. treatment				
Conc. (%)	**R1**	**R2**	**R3**	**R4**
5	21.74	27.32	31.16	23.3
10	28.2	34.3	39.3	27
15	42.1	38	45.3	23
20	59.21	41.12	46.3	26.37
25	58.03	66.29	53.11	47.07
4 hr. treatment				
5	25.27	31.06	43.1	23.05
10	35.25	42.19	30.36	37.28
15	30.64	48.11	54.34	31.35
20	50.04	58.03	67.12	49.07
25	71.21	68.42	57.13	75.94
6 hr. treatment				
5	68.13	21.77	23.19	43.03
10	40	55.15	70.03	46.06
15	74.31	69.02	39.63	51.12
20	83.15	60.32	64.19	76.22
25	96.11	81.13	88.01	68.07
24 hr. treatment				
5	63.02	75.08	72.8	60.3
10	74.08	69.08	82.31	58.17
15	71.51	83.12	87.07	65.32
20	67.02	89.11	87.26	79.17
25	104.67	99.1	85.16	80.4

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Table 1b):

2 hr. treatment				
Conc. (%)	**R1**	**R2**	**R3**	**R4**
5	19.51	19.25	20.52	12.84
10	28.04	27.01	50.23	13.16
15	41.32	33.15	44.08	26.41
20	45.04	29	37.02	53
25	54.18	49.15	55.39	41.28
4 hr. treatment				
5	21.17	44.15	17.09	20.31
10	30.11	27.09	15.04	52.2
15	30.05	47.1	32.12	51.05
20	48.32	63.35	53.06	59.27
25	76.39	68.18	46.08	54.41
6 hr. treatment				
5	65.03	24.06	40.03	26
10	51.77	57.02	35	48.23
15	74.31	69.13	27.06	51.02
20	69.11	72.01	77.02	55.02
25	69.14	87.34	85.07	78.13
24 hr. treatment				
5	61.3	66.13	57.4	72.1
10	69.32	85.14	54.35	71.19
15	79.52	68.13	63.03	82.32
20	60.12	85.19	79.11	86.06
25	99.6	77.05	86.2	97.36

Table 1c):

2 hr. treatment				
Conc. (%)	**R1**	**R2**	**R3**	**R4**
5	11.18	9.04	3.34	5.32
10	25.03	31	15	6.01
15	25.29	27.18	39.07	19.13
20	44.13	38.36	44.19	27.36
25	40.37	46.75	44.4	37.2
4 hr. treatment				
5	26.18	21.09	15.32	22.17
10	25.16	19.06	38.03	27.11
15	25.42	39.11	44.31	31.16
20	47.02	34.06	61.08	37.12
25	72.06	42.02	69.32	49.04
6 hr. treatment				
5	31	28.09	33.03	40
10	60.01	34.01	45.08	36.02
15	40.12	65.31	45.08	54.41
20	39.02	58.08	75.11	67.03
25	81.06	88.02	70.04	64
24 hr. treatment				
5	48.39	60.86	55.25	65.7
10	54.08	77.02	71.13	68.01
15	56.44	81.16	70.22	74.02
20	80.13	65.22	89.09	69.32
25	91.09	79.48	85.13	100.02

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Table 2: Repellency index of aqueous extracts of **a)** *Azadirachta indica* **b)** *Zingiber officinale* and **c)** *Annona squamosa* extracts at different exposure times (2-24 hr. post treatment) at indicated concentrations in a filter paper bioassay against whitefly *Bemesia tabaci.*

Table 2a):

2 hr. treatment				
Conc. (%)	**R1**	**R2**	**R3**	**R4**
5	24.02	15.86	15.86	25.22
10	27	38.1	30.2	28.1
15	32.08	45.4	31.16	28.32
20	26.75	21.03	44.19	68.15
25	49.24	59.28	53.33	47.55
4 hr. treatment				
5	25.09	29.19	43.12	19.32
10	32.12	27.31	49.08	24.41
15	53.08	32.17	47.14	31.33
20	57.03	40	65.13	45.16
25	74	59.14	67.11	62.03
6 hr. treatment				
5	46	34.03	60.01	26
10	51.09	59.05	34.02	53.16
15	41.17	65.02	44.11	55.26
20	72.41	57.08	65.12	61.31
25	68.12	90.09	79.04	88.07
24 hr. treatment				
5	57.75	71.3	71.42	68.29
10	59.35	78.11	71.64	67.34
15	57.19	85.32	72.08	75.13
20	69.13	88.32	82.19	77.36
25	90.77	79.46	85.14	98.75

Table 2b):

2 hr. treatment				
Conc. (%)	**R1**	**R2**	**R3**	**R4**
5	12.17	11.27	19.56	10.76
10	27.14	23.08	39.31	19.19
15	28.41	33.08	40.32	27.15
20	30.18	47.09	33.12	51.37
25	48.08	54.36	39.17	47.05
4 hr. treatment				
5	21.12	19.07	25.05	11.04
10	25.17	23.3	43.16	30.09
15	28.05	47.02	31	52.01
20	71.16	47.31	55.09	42.4
25	47.09	69.18	76.22	55.27
6 hr. treatment				
5	25.02	33	43	23
10	29	42.01	60.03	45
15	41.23	64.08	46.04	54.09
20	73.09	44.05	50	68.18
25	79.15	68.06	63.28	85.43
24 hr. treatment				
5	57.75	60.39	63.66	56.12
10	53.12	68.16	77.25	70.19
15	59.21	85.05	66.03	74.15
20	64	72.02	80.03	82.01
25	92.4	79.05	85.5	76.13

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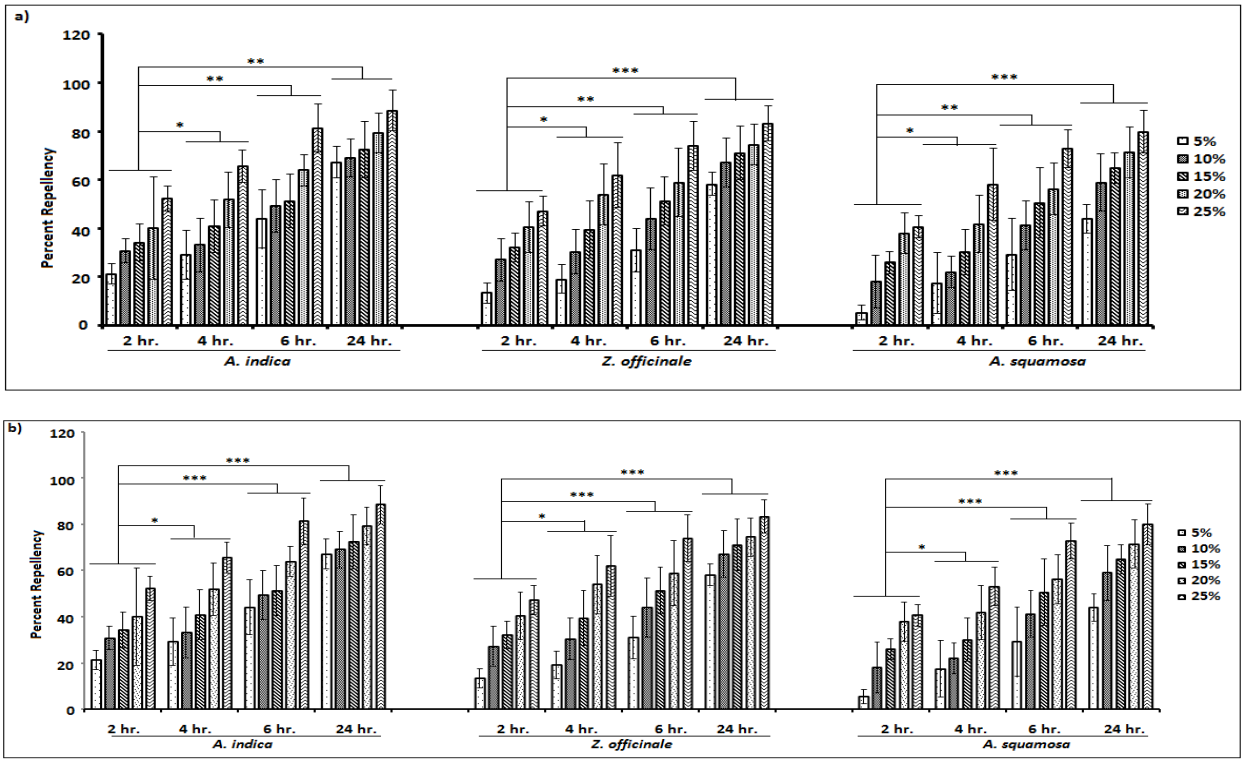
Table 2c):

2 hr. treatment				
Conc. (%)	**R1**	**R2**	**R3**	**R4**
5	6.11	8.87	1.32	5.08
10	22	31.03	13.06	6.15
15	22.04	26.18	32.31	23.35
20	43	36.02	46	27
25	43.53	42.28	33.75	43.2
4 hr. treatment				
5	25.14	30.21	3.33	11.34
10	30.08	21	14.04	23
15	41.12	25.19	34.29	20.08
20	32.15	54.3	49.19	31.36
25	13. 06	11.01	9.08	20.02
6 hr. treatment				
5	26	29	49	13.02
10	44.06	29.43	38.15	53.32
15	56.01	50.07	65.13	31.11
20	50.15	59.07	70.02	46.12
25	79.13	72.17	62.25	78.01
24 hr. treatment				
5	49.79	44.09	46.02	35.66
10	74.01	49	50.03	63
15	73.14	58.04	66.12	62.02
20	57.31	82.15	71.09	75.13
25	78.02	86.11	87.15	68.25

Figure 1 The average percent repellency of **a)** methanolic and **b)** aqueous extracts of *Azadirachta indica*, *Zingiber officinale and Annona squamosa* at indicated times of treatment and concentration. Results are expressed as mean± S.D of four replicates; p values were calculated in graph pad prism version 8.1.0 by performing one way ANNOVA by comparing average repellency values between varying treatment times; where *p≤ 0.05, **p≤0.01 and ***p≤0.001.



4. DISCUSSION

The above results clearly decipher that the extracts possess a significant repellency potential towards the pest. The methanolic as well aqueous extracts of *Azadirachta indica* showed the highest percent repellency followed by *Zingiber officinale* and *Annona squamosa* at all concentrations and time durations of treatment. These results are in accordance with some previous studies which reported that *Azadirachta indica* extract was highly effective as repellent on red pumpkin beetle [17]**.** *A. indica* seeds are also reported to have highest percentage repellency/ repellency indices against adult whiteflies and *Varroa destructor* (varroa mites) [18, 19]. Though a recent study which reported that certain neem based insecticide formulations like Azadirachtin A/B and/or neem oil proved much efficient in suppressing green peach aphid, *Myzus persicae* however the same couldn't show efficient repelling potential under standard spray conditions [20]. This could be due to the fact that the study employed individual constituents against aphid and it indicates that some other compound/s from the crude mixture will be playing a repellent role. Nevertheless, Azadirachtin a major constituent of *A. indica* is one of the potent repellents to many agricultural pests [21] besides neem oil which also has many repellent properties against anopheline and culicine mosquitoes [21-23].

Previously, *Z. officinale* besides some other plants has been reported to possess irritant and repellent effects against the malarial vector *Anopheles gambiae* **[24].** Ginger oil has also been reported to show significant repellency of *Bemisia argentifolii* infesting tomato plants [25]; however a desirable level of repellence couldn't be observed as higher concentrations of oil lead to leaf wilting. Likewise, essential oil from *Z. officinale* is also reported to show significant repellent effects on some mosquito species [26].

A. squamosa seed extract fractions are also reported to possess strong repellent and insecticidal properties against *Ceratitis capitata* (Wiedemann) [27]. Further A. squamosa seed extracts and/or essential oil has been reported to show significant repellent and larvicidal activities against rusty grain beetle *Trilobium castaeneum* [28, 29].

Taken together, the study reports a comparative pest repellent potential of three medicinal plants, using two solvents systems while extraction, however the effect of solvent didn't seem affecting the activity to a significant level. While this study is the first study from India comparing the repellent activities of these plants against *Bemisia tabaci*, further studies however are needed to isolate and characterize the active constituents which could be used in green house and/or fields to assess the efficacy in natural environmental conditions.

5. CONFLICT/S OF INTEREST

We declare no conflict/s of interest related to this work.

6. CONCLUSION

Finally it can be concluded that the repellant effect of the test plant extracts make them potential materials for use in a comprehensive integrated pest management program for the subject pest**.**

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