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EFFECTS OF BOTANICAL OILS ON *APHIS CRACCIVORA* Koch (HOMOPTERA: APHIDIDAE)

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ABSTRACT

In the present study, five botanical oils namely sesame, castor, groundnut, pongamia and black cumin were tested against the adult of *Aphis craccivora* Koch to evaluate the toxic and repellent effects under laboratory conditions (25 ± 5°C, 65-75% RH). Four concentrations (1.5, 2.0, 2.5 and 3.0%) along with on untreated control were utilized for the toxic and repellent study. Results indicated that all the botanical oils had different levels of toxic and repellent effects and found effective against the aphid. Among the tested botanical oils, the highest (40.50%) mortality was found in sesame oil while the lowest (30.50%) in black cumin oil against the aphid. The lowest LD_{50} values for sesame oil were found as 28.093, 4.529, 0.945 and 0.179 % at 12, 24, 36 and 48 HATs, respectively. Mortality percentages were dose dependent and proportional to the hours after treatments. The highest LD_{50} values were observed as 586.05, 14.860, 2.856 and 2.373% at 12, 24, 36, and 48 HATs, respectively for black cumin oil. Sesame oil also showed the highest repellent effect (69.00%) i.e., repellent class IV among all the botanical oils applied. On the contrary, black cumin oil showed the lowest repellent (33.33 %) effects, i.e., repellent class II. The order of toxicity was found as sesame oil > castor oil > groundnut oil > pongamia oil > black cumin oil considering all the concentration and efficacy. Therefore, among the tested oils sesame oil might be tested in field condition against the *A. craccivora*.

Keywords: Toxicity, repellenency, botanical oils, *Aphis craccivora*.

INTRODUCTION

The country bean, *Lablab purpureus* Lin. (Leguminosae: Papilionaceae), is an important vegetable-cum-pulse crop grown everywhere in Bangladesh (Rashid 1999). It is grown usually in winter season but recently, several photo-insensitive summer varieties are developed which help to promote the cultivation of country

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beans year-round. About 1, 44,050 metric tons of country beans are produced from 2.0881 hectares of land per year in Bangladesh (BBS 2019). During cultivation, the crop faces various problems including insect pests (Rashid 1999). Insect pests, which cause huge losses to bean crops, are serious problems worldwide. In Bangladesh, over 30 different species of arthropods have been reported to attack country bean crop, although only a few occur regularly and cause economic damage (Islam 1999).

The bean aphid, *Aphis craccivora* Koch (Homoptera: Aphididae) is the most destructive and cosmopolitan pests (Madahi *et al.* 2013). Both the nymphs and adults of *A. craccivora* suck sap from the tender part of plant and cause up to 10-90% yield loss (Akhtar *et al.* 2010, Razaq *et al.* 2011). It directly damage leaves, pods and other aerial tissues which tends the young seedlings succumb to death while mature plants show symptoms such as stunting growth, crinkling and curling of leaves, pods and finally cause yield reduction. Indirectly it transmits plant viruses such as mosaic virus (Kamphuis *et al.* 2012). They also secrete honeydew causing the growth of sooty mould fungus which inhibits the photosynthesis process (Singh *et al.* 2014). Thus an appropriate management strategy is needed against this insect pest.

To protect crops, growers in Bangladesh often apply synthetic chemical insecticides. It provides quick and adequate control for the time being, but is expensive and leaves long-lasting residues over the exposed surface of the crops, in soil and water (Hussain 1989). Besides, due to other problems such as health hazards, undesirable side effects, development of pest genotypes resistant to pesticides, resurgence and upset of pests and environmental pollution (Nas 2004), there is renewed interest in the application of botanical pesticides for crop protection (Pedigo and Lewis 2002). Hence, researchers and scientists all over the world are now trying to adopt alternatives to insecticides to protect the crop from insect pests (Rajappan *et al.* 2000, Aziz *et al*. 2018). A large number of plant products have been used successfully for controlling various pests in field and laboratory conditions (Bajpai & Sehgal 2000, Pedigo & Lewis 2002). Botanical products like tobacco extract, neem oil and extract, which can be easily and cheaply collected in rural Bangladesh, have been found promising and useful for pest control (Roy *et al*. 2005). Essential plant oils demonstrated high insecticidal activity against insect pests (Khater 2012). Therefore, the present study was undertaken to evaluate the effectiveness of five botanical oils viz., sesame, castor, groundnut, pongamia and black cumin against bean aphids.

MATERIALS AND METHODS

The experiment was carried out in the laboratory maintained at 25 ± 5°C, 65-75% RH in the Department of Entomology, of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during the period from September 2019 to January 2020.

Collection and rearing of tested insect: Bean aphids (*Aphis craccivora*) were collected from the infested bean plants (*Lablab purpureus* L.) of HSTU research field. Twigs harboring *A. craccivora* colonies were brought to the laboratory. Afterwards, aphids were gently removed from the bean twigs with the help of soft camel hair brush and were released on the fresh bean twigs kept in rectangular jars (14 cm x 10.5 cm x 30 cm) in the laboratory. The jars were covered with a piece of cloth and fastened with rubber bands to prevent insect's escape. Newly hatched crawlers were collected from the laboratory culture and were placed on bean twigs (*L. purpureus*) for mass culture. These processes were continued up to the experimental requirement.

Identification of adult *A. craccivora*: Usually aphid species are identified on the basis of their morphological characteristics (Emden & Harrington 2007). It was identified by using taxonomic key (Blackman & Eastop 2000). The apterae adults of *A. craccivora* are small, soft- bodied and has specialized piercing with sucking mouthparts which are used to suck the juice from plants and always shiny black without wax and approximately 2 mm long.

Tested botanical oils and dose preparation: Available of five botanical oils namely, *Sesamum indicum (*sesame), *Ricinus* communis (castor), *Arachis hypogaea* (ground nut)*, Pongamia pinnata* L. (karanja) and *Nigella sativa* (black cumin) were collected from the local market of Dinajpur town. Four concentrations (1.5, 2.0, 2.5, and 3.0%) were prepared with distilled water added one drop of Tween-20 by using micro pipette (Single channel micro pipette, by Dragon lap China, model: C40038142). Pilot experiments were done prior to select the appropriate doses.

Toxicity test (Leaf-dipped method): Insect bioassays were performed in the laboratory (25 ± 5°C, 65-70% RH) to determine the direct toxicity and repellent effects on *A. craccivora*. Fresh young leaves were collected from country bean plant in the experimental field. The test was performed by leaf-dip method (Yasmin *et al*. 2017) where 5 cm length leaf was put inside the Petri dish (150 mm). Leaves were dipped in assigned tested oils for 30-50 seconds containing each concentration (1.5, 2.0, 2.5, and 3.0%) and then the leaves were air-cured for 20-30 min so that excessive water dried away. Each leaf was placed separately in each Petri dish (150 mm). Five pair of adult aphids were released on each leaf twig with the help of a camel-hair brush. Three replications were maintained for each concentration. Control petri-dishes were untreated. Adult mortality was recorded at 12, 24, 36 and 48 hour after treatment (HAT). Observed insect mortalities (i.e., original data) were corrected following the procedures as described by Abbott (1987):

$$P - \frac{P' - C \times 100}{100 - C}$$

Where, *P* = Percentage of corrected mortality, *P'* = Observed mortality (%), C = Mortality (%) at control.

Insect repellency test: The repellency test was conducted according to the method described by Talukder and Howse (1994). Petri-dishes (120 mm diameter), filter papers (Whatman No. 40) were cut in two half and 1.0 ml of each concentration of five botanical oils was applied to a half filter paper uniformly with a pipette. The treated half were then air-dried and attached with the untreated half with a cello-tape. Precautions were taken so that attachment could no interfere with the free movement of insect from one half to another but distance between the filter paper segments remained sufficient to prevent seepage of test samples from one half of circle to another. Each filter paper was then placed in a Petri-dish and 5 pair of adults was released there. Three replications for each concentration of botanical oils were maintained with untreated control. Number of insects on each portion was counted at two hour intervals up to the 10^{th} hour. The data were expressed as percentage repulsion (PR) by the following formula: [PR (%) = (Nc-50) × 2]. Where, Nc = the percentage of insects present in the control half. Positive (+) values expressed repellency and negative (-) values attractancy. The average values were then categorized according to the following scale of McDonald *et al*. (1970).

Statistical analysis: The collected data were analyzed using Completely Randomized Design (CRD) through MSTAT-C program. The treatment mean values were compared by Duncan's New Multiple Range Test (DMRT). The median lethal values (LD_{50}) were determined by Probit analysis. Graphical works were done by Microsoft excel program.

RESULTS

Direct toxic effect of oils on *A. craccivora:* The accessible toxicity of botanical oils, doses and interactions effects against *A. craccivora* are shown in the Tables 1. The interaction effects of oils, doses and time indicated that there was a significant different among the toxicity of the botanical oils. Average value showed the highest mortality (55.83%) in sesame oil at 3.0% dose. Conversely, in untreated control, 2.23% mortality was recorded as the lowest among the treatments. All tested botanical oils showed different level of toxicity effect on bean aphid but sesame oil was the most effective against bean aphid.

Table 1. Interaction of effects of oils, doses and times against bean aphid at different HATs

Treatments (oils)	Doses (%)	Mortality (%) at different HATs				Average mortality (%)
		12HAT	24 HAT	36 HAT	48 HAT	
Sesame	1.5	13.33 a	33.33 a-c	56.67 a-d	80.00 bc	45.83 c-e
	2.0	16.67 a	36.67 a-c	60.00 a-c	83.33 bc	49.17 bc
	2.5	16.67 a	40.00 ab	63.33 ab	86.67 ab	51.67 ab
	3.0	20.00 a	43.33 a	66.67 a	93.33 a	55.83 a
Castor	1.5	13.33 a	30.00 a-c	46.67 de	76.67 cd	41.67 d-g
	2.0	16.67 a	33.33 a-c	50.00 cd	76.67 cd	44.17 c-f
	2.5	16.67 a	33.33 a-c	53.33 b-d	80.00 bc	45.83 c-e
	3.0	20.00 a	36.67 a-c	56.67 a-d	83.33 bc	49.17 bc
Groundnut	1.5	13.33 a	30.00 a-c	46.67 de	70.00 de	40.00 e-g
	2.0	13.33 a	30.00 a-c	46.67 de	76.67 cd	41.67 d-g
	2.5	16.67 a	33.33 a-c	50.00 cd	76.67 cd	44.17 c-f
	3.0	16.67 a	33.33 a-c	53.33 b-d	80.00 bc	45.83 c-e
Pongamia	1.5	13.33 a	23.33 cd	46.67 de	66.67 ef	37.50 g
	2.0	13.33 a	26.67 bc	50.00 cd	70.00 de	40.00 e-g
	2.5	13.33 a	30.00 a-c	53.33 b-d	76.67 cd	43.33 d-f
	3.0	16.67 a	33.33 a-c	60.00 a-c	76.67 cd	46.67 b-d
Black cumin	1.5	10.00 a	13.33 d	36.67 e	60.00 f	30.00 h
	2.0	13.33 a	30.00 a-c	46.67 de	66.67 ef	39.17 fg
	2.5	13.33 a	30.00 a-c	46.67 de	70.00 de	40.00 e-g
	3.0	13.33 a	33.33 a-c	50.00 cd	76.67 cd	43.33 d-f
Control	0	0.00 b	0.00 e	3.33 d	3.33 e	2.23 d
LSD		9.656	11.98	10.20	8.254	4.988
CV %		49.07	28.83	14.95	8.24	8.69
P-value		0.00	0.00	0.00	0.167	0.041

HAT= Hours after treatment. Mean followed by different letter(s) are significantly different by DMRT at 5% level of probability

Probit analysis for direct toxic effect of botanical oils against *A. craccivora*: The calculated LD_{50} values, 95% confidence limits and Chi square values are presented in Table 2. The lowest LD_{50} value of sesame oil was 28.09% at 12 HATs which indicated the most toxic followed by castor oil (87.78%). Conversely, the highest LD_{50} of black cumin (586.05%) showed the least toxic among the treatments. Similarly, sesame oil was the most toxic (4.53%) as compared to other treatments after 24 HATs. At 36 HAT showed similar result where sesame oil showed the highest toxicity (0.95%) followed by castor oil (1.86%) while black cumin oil (2.86%) showed the lowest toxicity among all the treatments. Again, at 48 HAT sesame oil aqua showed the most toxic (0.18%), as compared to other treatments applied. It was observed that the LD_{50} values of sesame oil provided maximum toxicity of 28.09, 4.53, 0.95 and 0.18 at 12, 24, 36 and 48 HATs, respectively. The chi-square (χ^2) values were insignificant at 5% level of probability among botanical oils at different HATs and mortality data did not show any heterogeneity.

Table 2. Relative toxicity (by probit analysis) of different botanical oils treated against bean aphid after 12, 24, 36 and 48 HATs

Treatments (Oils)	No. of insect used	LD_{50} values (%)	95 % fiducially limits		χ^2 values with 4df	Regression equations
			Lower	Upper		
12 HAT						
Sesame	30	28.09	1.93	40790.3	8.32	Y= 0.8797x + 3.7351, R^2 = 0.9036
Castor	30	87.78	5.55	1.39	6.54	Y= 0.4332x + 3.9586, R^2= 0.7835
Groundnut	30	110.59	2.31	5.30	5.16	Y= 0.7088x + 3.7204, R^2 = 0.7835
Pongamia	30	249.76	8.95	6.97	0.113	Y= 0.099x + 3.8515, R^2 = 0.9895
Black cumin	30	586.05	1.47	2.34	5.85	Y = 0.5083x + 3.6632, R^2 = 0.7376
24 HAT						
Sesame	30	4.53	0.871	23.53	5.13	Y= 0.8603x + 4.4092, R^2 = 0.9998
Castor	30	4.62	1.92	11.09	1.065	Y= 0.5592x + 4.3785, R^2 = 0.8645
Groundnut	30	8.72	0.38	202.26	5.08	Y= 0.315x + 4.4135, R^2 = 0.7835
Pongamia	30	14.74	7.62	28,524.6	4.18	Y= 0.9913x + 4.0874, R^2 = 0.9995
Black cumin	30	14.86	5.65	3.92	3.62	Y= 2.1754x + 3.6121, R^2 = 0.7756
36 HAT						
Sesame	30	0.95	0.11	8.00	2.38	Y= 0.8422x + 5.0153, R^2 = 0.9582
Castor	30	1.86	1.03	3.36	0.215	Y= 1.0394x + 4.7112, R^2 = 0.9147
Groundnut	30	1.95	0.98	3.89	8.96	Y= 0.8467x + 4.7588, R^2 = 0.9763
Pongamia	30	2.37	0.72	7.83	8.76	Y= 0.5352x + 4.7991, R^2 = 0.8224
Black cumin	30	2.86	1.37	5.95	0.26	Y= 1.0215x + 4.5322, R^2 = 0.8549
48 HAT						
Sesame	30	0.18	1.08	315.99	0.18	Y= 1.9927x + 5.4289, R^2 = 0.8713
Castor	30	0.54	8.34	3.56	0.35	Y= 0.7084x + 5.5805, R^2 = 0.8286
Groundnut	30	0.65	6.92	6.18	0.13	Y= 0.5352x + 4.7991, R^2 = 0.8224
Pongamia	30	0.97	0.28	3.41	0.14	Y= 1.1227x + 5.2305, R^2 = 0.8996
Black cumin	30	2.37	0.72	7.83	8.76	Y= 1.5296x + 4.9704, R^2 = 0.9574

Probit mortality of insectHAT = Hours after treatment. Values were based on five concentrations, three replications of 10 insects each. $\chi 2$ = Goodness of fit. The tabulated value of $\chi2$ is 5.99 (d.f = 2 at 5% level)

Probit regression lines for direct toxic effect of oils against *A. craccivora*: The relationship between probit mortality of insect and log doses of the tested botanical oils are presented in Fig. 1. The insect mortality rate showed positive correlation with the doses in all treatments. The probit regression lines of botanical oils showed a clear linear relationship between probit mortality and their log doses and the regression lines become sleeper as doses increased because the *A. craccivora* were treated with more toxic for the same period with higher doses.

Repellent effect of botanical oils against *A. craccivora*: The repellent effect of five botanical oils, their doses and interaction effects of bean aphid were shown in Table 3. Sesame, castor, groundnut, pongamia and black cumin oils showed statistically different repellent effects against bean aphid. Of the tested botanical oils, the highest repellent effect was found in 3.0% sesame oil (84.0%) but the lowest in 1.5% black cumin oil (22.67%). The repellency class of different botanical oils at different concentrations level varied between II to V. From the above results it was observed that all the tested botanical oils repelled the bean aphid but sesame oil was the most effective.

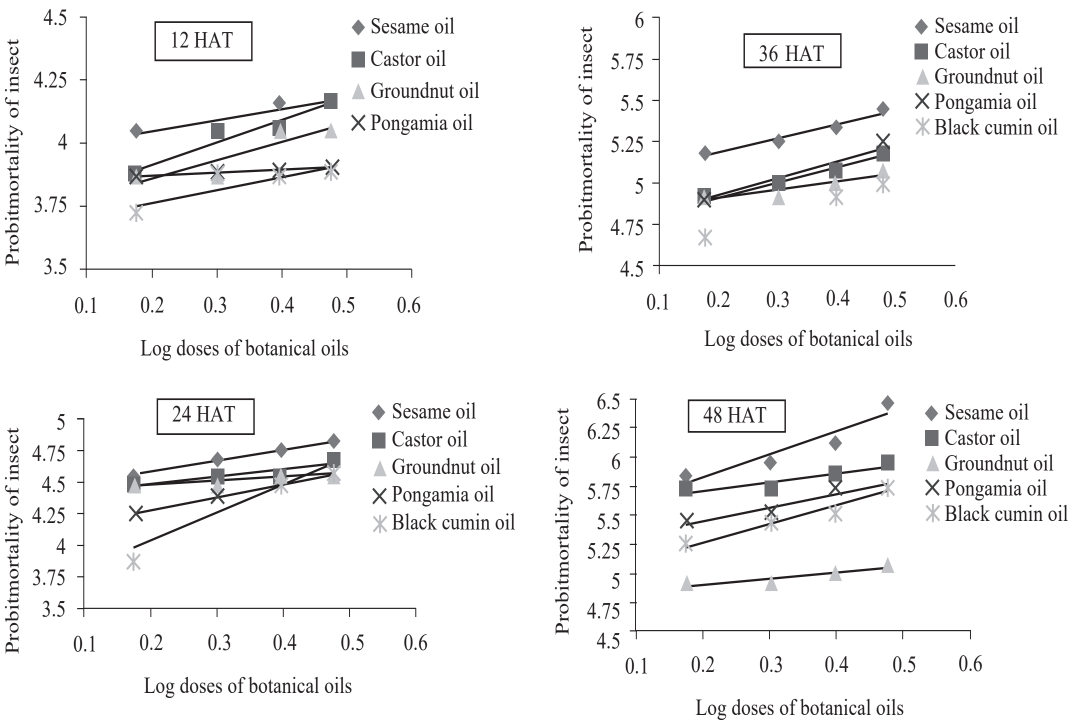

Fig. 1. Relationship between probit mortality and log doses of different botanicals on bean aphid at 12, 24, 36 and 48 HATs.

Table 3. Repellent effect of different botanical oils and doses against bean aphid at different HATs (interaction of botanical oils, doses and times)

Treatments (oils)	Dose (%)	Repellency (%) at different HATs					Mean repellency	Classes
		2	4	6	8	10		
Sesame	1.5	93.33 a	86.67 a	40.00 ab	46.67 ab	60.00 a-e	65.33 ab	IV
	2.0	46.67 b-d	80.00 ab	66.67 ab	60.00 ab	53.33 a-e	61.33 a-c	IV
	2.5	80.00 ab	66.67 a-d	33.33 ab	53.33 ab	93.33 a	65.33 ab	IV
	3.0	93.33 a	86.67 a	80.00 a	73.33 a	86.67 ab	84.00 a	V
Castor	1.5	60.00 a-d	53.33 a-d	40.00 ab	46.67 ab	73.33 a-d	54.67 b-e	III
	2.0	53.33 b-d	46.67 a-d	40.00 ab	60.00 ab	80.00 a-c	56.00 a-d	III
	2.5	66.67 a-c	60.00 a-d	46.67 ab	53.33 ab	66.67 a-e	58.67 a-c	III
	3.0	80.00 ab	73.33 a-c	66.67 ab	53.33 ab	60.00 a-e	66.67 ab	IV
Ground nut	1.5	46.67 b-d	40.00 a-d	33.33 ab	33.33 ab	40.00 cde	38.67 b-f	II
	2.0	40.00 b-d	33.33 b-d	40.00 ab	33.33 ab	46.67 b-e	38.67 b-f	II
	2.5	53.33 b-d	46.67 a-d	40.00 ab	46.67 ab	53.33 a-e	48.00 b-f	III
	3.0	60.00 a-d	53.33 a-d	46.67 ab	40.00 ab	60.00 a-e	52.00 b-f	III
Pongamia	1.5	26.67 cd	20.00 d	26.67 b	20.00 b	33.33 de	25.33 ef	II
	2.0	40.00 b-d	33.33 b-d	26.67 b	33.33 ab	40.00 c-e	34.67 c-f	II
	2.5	46.67 b-d	40.00 a-d	33.33 ab	26.67 ab	46.67 b-e	38.67 b-f	II
	3.0	46.67 b-d	40.00 a-d	40.00 ab	46.67 ab	46.67 b-e	44.00 b-f	III
Black cumin	1.5	20.00 d	26.67 cd	20.00 b	20.00 b	26.67 e	22.67 f	II
	2.0	33.33 cd	26.67 cd	20.00 b	26.67 ab	33.33 de	28.00 d-f	II
	2.5	40.00 b-d	33.33 b-d	26.67 b	20.00 b	40.00 c-e	32.00 c-f	II
	3.0	40.00 b-d	33.33 b-d	33.33 ab	26.67 ab	40.00 c-e	34.67 c-f	II
LSD		34.35	41.53	40.87	40.87	38.35	25.24	
CV %		39.03	51.36	61.91	60.40	43.03	32.22	
P-value		0.00	0.00	0.00	0.00	0.00	0.00	

HAT= Hours after treatment. Mean followed by different letter(s) aresignificantly different by DMRT at 5% level ofprobability.

DISCUSSION

Results from the present study indicated that all the tested botanical oils had promising toxic effects against the *A. craccivora* in laboratory conditions. However, the black cumin oil offered promising toxicity by applying the highest dose of 3.0% against the aphid (Table 1). Our present findings are also in close agreement with those of Yasmin *et al*. (2017). They found that botanical oils proved toxic as insecticide against the adult *A. craccivira* under laboratory conditions. Present findings were also comparable with those of Bahar *et al.* (2007) and Pinto *et al*. (2013). They experienced that the eucalyptus, mehogony oils reduced aphid population but different among various ecological circumstances like field, net-house and laboratory. The results are in agreement with the findings of Veena *et al*. (2017) who compared the effectiveness of plant oils for increasing the efficacy of insecticides and acaricides against chilli mite. They used castor oil, pongamia oil, sesamum oil and mustard oil at different intervals of time. Castor oil emerged as the best material for increasing the efficacy of pesticides against sucking pests of chilli. Thein *et al*. (2019) tested synergistic toxicity of sesame and clove essential oils against the *Callosobruchus maculatus* adults, through residual contact and fumigation tests. Sesame oil could be an important implication in synergisms with clove oil to reduce cost of application for controlling *C. maculatus* in storage. Diverse plant products were evaluated by many researchers and found potential against aphids, such as orange peel *(Citrus sinensis*), bitter gourd (*Momordica dioica*), garlic (*Allium vineale*), marigold, hot pepper (*Capsicum frutescens*) and tobacco (*Nicotiana tabacum*) to wheat aphid (Iqbal 2011); garlic bulbs *(A. sativum*), endod (*Phytolacca dodecandra*) and neem seeds (*Azadirachta indica*) to pea aphids, *Acyrthosiphon pisum* (Harris) (Megersa 2016). Dennis (1990) reported that coating legume seeds with oils extracted from plant is effective in bruchid control. Reena and Sinha (2012) found that *P. pinnata* seeds extractsat of 5.0% concentration exhibited more than 50% first instar larval mortality and more than 65% third instar larval feeding deterrent against *Helicoverpa armigera,* American bollworm. Fenigstein *et al*. (2001) tested the effects of five economically important vegetable (seed) oils, peanut, cotton seed, castor, soybean and sunflower, on adult and immature stages of the sweet potato whitefly (*Bemisia tabaci* (Gennadius) [Hemiptera: Aleyrodidae]. Peanut oil was the most effective among the tested oils.

Again, present results are also in line with Lin *et al*. (2009). They reported that sugar apple (*Annona squamosa*), an edible oil extracted from tropical fruit was also promising against the cotton aphid, *A. gossypii* Glover infested on melon plant. Again, *Acacia concianna* extract was found as the most toxic against sugarcane woolly aphid, *Ceratovacuna lanigera* Zehnter (Patil & Chavan, 2009). Some plant origin oils have broad spectrum insecticidal activities found against many destructive pests those affecting insect nervous and defense systems (Isman 2000, Ketoh 2004). Besides, physiology of the destructive insects is arrested due to biological activity of oils which resulting accelerated death (Schoonhoven, 1978).

From the Present findings it was proved that all the tested botanical oils significantly repelled the bean aphid but sesame oil exibited as the most effective (Table 3). Present results are comparable with Suthisut *et al.* (2011). They experienced that from *Alpinia conchigera* and *Curcuma zedoaria* extracted essential oils with the highest concentration of 314.56 × 10^{-3} μl/cm^2 repelled *S. zeamais* adults, with > 87% of the insects found on the untreated filter paper. Besides, aphid populations suppressed more effectively when it was applied in combination with neem and eucalyptus, *Eucalyptus globulus* (Myrtaceae) leaves extract (Ali *et al.* 2015, Manzoor *et al.* 2015, Shah *et al*. 2017). These chemical compounds might associated with deterrent, repellent and anti-feeding actions against *A. craccivora*, *B. brassicaea* (Homoptera: Ahididae) but seems harmless or tolerated by natural enemies as well (Katsvangwa and Chigwaza 2004).

CONCLUSION

Botanical oils used in the present study had direct toxic effect on bean aphid, *A. craccivora* which can be used as safe pesticides. Uses of these botanical pesticides have a great economic and environmental importance. Sesame oil showed the highest toxic and repellent effects against the *A. craccivora,* among the tested oils. Based on efficacy of the tested botanicals, sesame oil can be used at field level in integration with other IPM components rather than its sole application. However, further studies are needed to be conducted to isolate and evaluate the compound with its mode of action of these botanicals.

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