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Efficacy of *Azadirachta indica* (A. Juss) seed powder water extract against A*ulacuspis tubercularis* New Steed (Homoptera: Diaspididae) on mango (*Mangifera indica L.*) In East Wollega, Ethiopia

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ABSTRACT: Mango (*Mangifera indica L.)* is a fleshy stone fruit belonging to the genus *Mangifera*. Among the many tropical fruits, mango has been identified as the most important and considered to be the king of fruits from a socio-cultural, commercial and environmental point of view and grown in over 100 countries including Ethiopia. However, many biotic and abiotic factors limit its production and productivity across the globe. *Aulacuspis tubercularis Newsteed* (Homoptera: Diaspididae) commonly known as white mango scale is a serious insect pest of mango in Ethiopia. Though A. tubercularis introduced to Ethiopia almost a decade ago, technologies towards its control is almost nil or few which enabled the pest to invade the whole country where mango is grown to the extent of causing 50-100% crop losses. The current study was conducted to know the efficacy of *Azadirachta indica* (A. Juss) seed powder water extract in the management of *A. tubercularis* under field condition. Field experiments were conducted at Uke and Arjo Gudetu in western Ethiopia. The treatments were different spray concentrations. (0.05, 0.1 and 0.15mg/ml of water). The treatments were applied 3 times at 10 days interval after complete infestation was observed. The experiment was laid out in a randomized complete block design in four replications. Mortality count was done 10 days after 1st, 2nd and 3rd treatment applications. The results obtained revealed that water extracts of *A. indica* seed powder at 0.15 concentration significantly ($p<0.05$) reduced the population of *A. tubercularis* at both experimental sites. Crawlers and males were more affected than the females. Hence, the use of *A. indica* seed water extract can be recommended for the management of *A. tubercularis.*

Key words/phrases: ***Aulacuspis tubercularis****,* ***Azadirachta indica****,* **Efficacy, Management, Seed powder water extract**

INTRODUCTION

Mango (*Mangifera indica* L.) is a fleshy stone fruit belonging to the genus *Mangifera*. It is commercially growing in more than 100 countries (Hernandez *et al*., 2011), of which more than 65 countries produce each more than 1,000 metric tones' annually (FAOSTAT, 2018). Mangoes are one of the most delicious and nutrient-dense fruits, rich in vitamins A, C, and D and are known worldwide as the king of fruit (Pradeep *et al*., 2011). Among the many tropical fruits, mango has been identified as the most important from a socio-cultural, commercial and environmental point of view (Anshuman *et al*., 2015). The total world production was over 55.38 million tones, with a production area of 5.75 million hectares (FAOSTAT, 2018) which played an integral part in the lives of many, not only by being rich in nutrients, but also as a source of livelihood for millions of peoples particularly in the tropics.

Mango is one of the most widely grown fruit crops in Ethiopia preceded only by banana in terms of economic importance. Most of the productions come mainly from the Rift Valley, western and south western Ethiopia (Tewodros Bezu *et al*., 2019). The annual mango production in Ethiopia is 133,704.93 tones with land coverage of 19,497.92 ha and its production is 6.86 tones ha^{-1} (CSA, 2019), which accounts for 0.18% of the world production (FAOSTAT, 2018). Mango production in Ethiopia is at small scale level with primary purposes of family consumption and local markets. Mango production in Ethiopia is constrained by a number of factors of which damage by *Aulacaspis tubercularis* New stead (Homoptera: Diaspididae) is the most important (Temesgen Fita, 2014; Ofgaa Djirata *et al*., 2019). At high level of infestations *A. tubercularis* causes losses ranging from 50% to 100% (Kondo and Munoz-Velasco, 2009). *A. tubercularis* was first detected in Ethiopia in 2010 infesting mango plants in western parts of the country, East Wollega Zone from where it was distributed to different parts of the country (Temesgen Fita, 2014; Ofgaa Djirata *et al.*, 2019). Regardless of the importance of the pest in Ethiopia, efforts towards the management of the pest are minimal. Hence, the current study was conducted to see the efficacy of *A. indica* seed water extract under field condition.

MATERIALS AND METHODS

Description of the Study area

The experiments were conducted in East Wollega Zone (9°5′N 36°33′E) at Digga District, Arjo Gudatu kebele (9°02.225′N, 36°15.013′E) and at Guto Gida district Uke kersa Administrative kebele Uke site (9°18.908′N, 36°31.473′E) from March 2018 to May 2018. The districts were selected because of their representativeness in terms of mango producing agro-ecological zones. The ecological zones of the two districts are highlands (wet dega), wet midlands (weyna dega) and lowlands (wet kolla) (Table1). Guto Gida district receives high to moderate rainfall and represent high to low altitude areas, while Diga district represented midland and lowland ecologies. Elevation of Guto Gida district ranged from 1350 meters above sea level (m.a.s.l.) to 2900 m.a.s.l., while that of Diga district was from 1250 m.a.s.l.to 2300 m.a.s.l.

Table 1. Description of the main Agro-ecological zones of the study districts.

District	Agro-ecological zone	Altitude (m.a.s.l.)	Mean temp. (0°C)	Mean annual Rainfall (mm)
Guto Gida	Highland	2300-3200	12-18	2244.3
	Midland	1500-2300	18-25	2071.6
	Lowland	500-1500	>25	1516.9
Diga	Midland	1500-2300	18-25	1754.8
	Lowland	500-1500	>25	1663.6

Source: A Guideline for Development Agents on Soil and Water Conservation in Ethiopia (Hurni et al., 2016) and NMA of Ethiopia

A. tubercularis crawlers, adult males and females were identified in the School of Veterinary laboratory, Wollega University (9°.04′51.90′′N, 36°.34′57′′°E).

Plant material and extract preparation

Ripen *A. indica fruits* were collected from neem trees in Dire Dawa town. The seeds were washed thoroughly with water to remove any dirt or other undesirable accumulations on the kernels, which may reduce the efficacy of the final product. The outer coat of the seed kernels were removed from the fruits at its fresh stage before drying. The dehulled *A. indica* seeds were dried on plastic sheets placed on wooden benches for good circulation of air. The drying process was carried out under shade as *A. indica* chemicals degraded and lose their insecticidal value under sunlight. The *A. indica* seeds were collected 3 months earlier before the start of the experiment. The dried *A. indica* seed were gently grinded by electric grinder (Model SZJ-830 'S SAYONA Patirrier DELUXE COFFEE and SPICE GRINDER 220-240V 50-60HZ') to form fine powder in such a way that no oil comes out. The powder was kept in a cotton cloth bags which was soaked overnight in water and stored in a plastic bucket. The pouch was squeezed and the extract was filtered. Following the procedure of Eureka and Kaushik (2016) an emulsifier soap powder was added to the filtrate of *A. indica* seed powder water extracts. One milliliter of emulsifier was added to one liter of water to stick well to the sprayed leaf surface.

Experimental design and treatment application

Field experiment was carried out to evaluate the efficacy of water extracts of *A. indica* seed powder for the control of *A. tubercularis* on mango. The treatments were 0.05, 0.10 and 0.15 ml of the filtrates. Distilled water was used as a negative control for comparison. The concentrations were calculated using the following formula $C_1V_1=C_2V_2$, where C_1 and C_2 represent initial and final concentration, respectively and V_1 and V_2 represent initial and final volume, respectively (Kudom *et al.*, 2011).

The experiment was designed in a Randomized Complete Block design in four replications. An experimental field consisted of sixteen mango plants occupying an area of 32 m x 28 m was used. A plot size was 224 m^2. A plot consists of 1 mango plant. Spacing between plants and rows were 7m and 8m, respectively. Similar age and size mango plants were considered for the experiment. The mango plants used for the experiment were not treated with pesticides at least for the last two years. Cultural practices such as hand weeding and mowing were used for weed control. Before the application of the treatment to their respective plots (trees) calibration was done to know the amount of water to be used as the carrier. Accordingly, 3 l of water was used for each plant. A manually calibrated 'Knapsack Sprayer Thailand made (Jacto16 HD400) was used for treatment application. Treatment application started on April 20, 2018 when the infestation of *A. tubercularis* reached climax (almost 90-100% of the lower canopy leaves of the plants infested) and repeated every 10 days for three rounds. Spraying of the extract was carried out in the afternoon at 3:30 pm to reduce loss of the chemicals due to evaporation.

Data collection

A total of twelve mango leaves were sampled from top, middle and lower canopies of each tree 1 day, 3 days and 6 days after treatment application and kept in a paper bag, labeled and taken to the School of Veterinary laboratory, Wollega University. The number of dead adults and crawlers of *A. tubercularis* after spray were counted under dissecting microscope (WESCO®, Valencia, CA). The dead adults and crawlers were converted into percentage mortality. Pre- and post-spray counts of the crawlers and adults per leaf were also recorded from the sampled leaves and the reduction in infestation (efficacy %) was computed following Henderson and Tilton (1955) equation;

Percent Reduction =

$$1-\left(\frac{\text{"n" in treatment after treatment}}{\text{"n" in treatment before treatment}} X \frac{\text{"n" in control before treatement}}{\text{"n" in control after treatment}}\right)$$

Where, "n" is a number of *A. tubercularis* in the treatment (before and after) and in the control.

Any change in color and texture of leaves due to probable phytotoxicity of the tested *A. indica* seed powder water extract was recorded. Results of mortality were presented as percentage mortality, with correction for untreated (control) mortality using Abbott's formula (Abbott, 1925) as follows;

$$\text{Mortality\%} = \frac{\text{Observed mortality in the treatment} - \text{Mortality in control}}{100 - \text{Mortality in control}} x100$$

A. tubercularis was considered as died if there is a change in color (cloudy or blackish), dried and empty, and no movement of appendages when rubbed with fine brush.

Statistical analysis

The Mixed Procedure Repeated-measure was employed for analysis of variation between experimental units (Smith *et al*., 2017). A REML estimator for variance parameters was used as a method for fitting linear mixed models. Significant means (P<0.05) were separated using Tukey's Honestly Significant Difference (HSD) method (SAS Institute Inc., 2004). The data were then subjected to Probit (proc probit) analysis (Finney, 1947). Dose response mortality data were analyzed using linear regression analysis and the LC_{50} values for treatments were obtained (Robertson *et al*., 20070Busvine, 1971; Zar, 1999).

RESULTS AND DISCUSSION

Result of mortality of sessile *A. tubercularis* due to different concentrations of *A. indica* seed powder water extract at Uke and Arjo Gudetu site is shown in Table 2. Mortality of sessile *A. tubercularis* increases significantly (p<0.0001) with an increase in the concentration of *A. indica* seed powder water extract.

The highest mean percent mortality of *A. tubercularis* was recorded with 15% concentration, while the lowest was with 5% concentration at both sites.

Table 2.*Effect of *A. indica* seed powder water extract on mean (± SE) percent mortality of *A. tubercularis* at Arjo Gudetu and Uke sites.

Treatment concentration (%)	Frequency of treatment application	Experimental sites	
		Arjo Gudetu	Uke
5	3	62.92 ± 0.32c	64.73 ± 0.26c
10	3	79.34 ± 0.72b	75.30 ± 0.24b
15	3	86.79 ± 0.44a	83.34 ± 0.31a

**Means followed by the same letter (s) within a column are not significantly different from each other at 5% level, Tukey's studentized range test*

Table 5. *LC_{50} and LC_{95} of *A. indica* seed powder aqueous extract against *A. tubercularis* (n=360 each) at Arjo Gudetu and Uke sites.

Study Site	DAT	LC_{50} (µg/ml)	LL-UL	LC_{95} (µg/ml)	LL-UL	Slope ± SE	(X^2)
Uke							
	10	10.77	9.76-11.88	59.87	67.71-72.21	4.94±0.96	195.22
	20	4.20	3.63-4.85	66.71	46.64-95.41	5.43±0.93	398.16
	30	2.75	2.25-3.36	33.51	25.58-43.88	5.69±0.89	650.17
Arjo Gudetu							
	10	8.24	7.83-8.66	50.36	41.50-61-12	5.09±0.96	214.66
	20	3.95	3.54-4.41	26.78	22.47-31.93	5.58±0.89	594.48
	30	2.08	1.59-2.71	24.39	18.95-31.39	5.85±0.87	627.47

DATA=Days after treatment application; LC=Lethal Concentration; LL=Lower limit; UL= Upper Limit; LC_{50} and LC_{95} values are expressed as percentage (n=360); SE=Slope of the concentration-mortality regression line ± standard error; x^2 =Pearson's Chi-square value.

Implies that the x^2 values are significant by Tukey's HSD test at $p \leq 0.05$ levels and therefore a heterogeneity factor is used in the calculation of the confidence interval.

The essential extracts of *A. indica* seed powder water extract contact toxicity against *A. tubercularis* crawlers, adult females and males at Arjo Gudetu and Uke experimental sites are presented in Table 6. The mortality count was made at 10days after 1st 2nd and 3rd round treatments. The essential extracts of *A. indica* seed powder water extract against different stages of *A. tubercularis*; crawlers, adult males and females at Arjo Gudetu for 1st round treatments showed contact toxicity LC_{50} values of 6.12, 13.98 and 7.18 µg/ml, respectively. The contact toxicity of *A.indica* seed powder water extract against crawlers, adult males and females for 2nd round treatments were 3.68, 6.23 and 4.22 µg/ml. With the same activity the contact toxicity for the crawlers, adult males and females after 3rd round treatment showed LC_{50} values of 2.88, 3.90 and 2.91µg/ml, respectively. With the same activity the essential extracts of *A. indica* seed powder water extract against different stages of *A. tubercularis*; crawlers, adult males and females at Uke experimental site for 1st treatment showed contact toxicity LC_{50} values of 5.69, 28.07 and 8.77µg/ml, respectively. The contact toxicity of *A. indica* seed powder water extract against crawlers, adult males and females for 2nd round treatments were 3.59, 10.69 and 3.83µg/ml and for 3rd round treatments were 2.74, 6.20 and 3.27µg/ml, respectively. When the contact toxicity of *A. indica* seed powder water extract against adult female compared with the crawlers and male adults, the female adult was less affected by *A. indica* seed powder aqueous extract.

At both experimental sites the Chi-square values were significant at $P \leq 0.05$ level implies that the treatments were promising for management of *A. tubercularis.* The high Chi-square values in the treatments probably indicated the heterogeneity of the test population. Different concentration levels of aqueous extracts influenced sessile *A. tubercularis* mortality differently. At both experimental sites the control (distilled water) did not showed significant mortality, rather the mortality was natural death.

The log probit regression line calculated at Arjo Gudetu for 1st 2nd and 3rd round treatments were Y=1.83x+3.28 (X-0.56), Y=2.28x+3.64 (X-0.37) and Y=1.88x+4.39 (X-0.24), respectively (Figure 1), while the log probit regression line calculated at Uk for 1st, 2nd and 3rd round treatments were Y=1.125x +3.836 (X- 0.325), Y=1.053x +4.406 (X-0.203) and Y = 1.601x +4.354 (X- 0.219), respectively (Figure 2).

Table 6.The toxicity (LC_{50} and LC_{95}) of *A. indica* seed powder aqueous extract essential constituents on *A. tubercularis* crawlers, adult males and females 10 days after 1st, 2nd and 3rd round treatments (n=360) at Uke and Arjo Gudetu sites.

Study Site	Sex/Stage of WMS	DAT	LC_{50} (µg/ml)	LL-UL	LC_{95} (µg/ml)	LL-UL	Slope ± SE	(X^2)
Arjo Gudetu	Crawlers	10	6.12	5.57-6.52	28.31	22.97-34.87	5.38±0.94	3.84
	Female	10	13.92	12.29-15.75	100.42	59.88-168.38	4.69±0.98	0.22
	Male	10	7.18	6.55-7.86	40.56	30.37-54.15	5.21±0.95	4.36
	Crawlers	20	3.68	3.27-4.13	9.72	8.87-10.64	5.99±0.82	1.32
	Female	20	6.73	5.81-7.79	97.93	50.53-189-78	5.18±0.95	1.69
	Male	20	4.22	3.77-4.72	14.02	12.37-15.87	5.76±0.86	6.45
	Crawlers	30	2.88	2.39-3.47	7.08	6.48-7.73	6.33±0.77	1.43
	Female	30	3.90	2.95-5.15	68.39	36.54-127.99	5.47±0.94	1.89
	Male	30	2.91	2.39-3.54	10.97	9.75-12.33	6.13±0.86	3.70
Uke	Crawlers	10	5.69	4.64-6.96	125.80	53.85-293.91	5.24±0.95	0.13
	Female	10	28.07	17.73-44.43	1032.36	164.16-6491.82	4.50±0.97	0.15
	Male	10	8.77	7.49-7.25	263.91	81.69-852.49	5.02±0.96	0.00
	Crawlers	20	3.59	3.06-4.22	15.14	13.07-17.53	5.87±0.88	2.12
	Female	20	10.69	8.62-13.25	901.25	113.62-7148.55	4.94±0.96	1.24
	Male	20	3.83	3.02-4.85	41.80	27.46-63.63	5.56±0.94	0.52
	Crawlers	30	2.74	2.23-3.37	8.21	7.39-9.12	6.22±0.79	0.35
	Female	30	6.20	5.20-7.39	114.93	53.30-247.81	5.21±0.96	0.43
	Male	30	3.27	2.71-3.94	15.05	12.85-17.61	5.92±0.89	1.04

*WMS=White mango scale (A.tubercularis); DAT= days after treatment; LC=Lethal Concentration; LL=Lower Limit; UL=Upper Limit; LC_{50} and LC_{95} values are expressed as percentage (n=360); SE: Slope of the concentration-mortality regression line ± standard error; x^2=Pearson's Chi-square test. *The X^2 values are significant by Tukey's HSD test at $p \leq 0.05$ levels.*

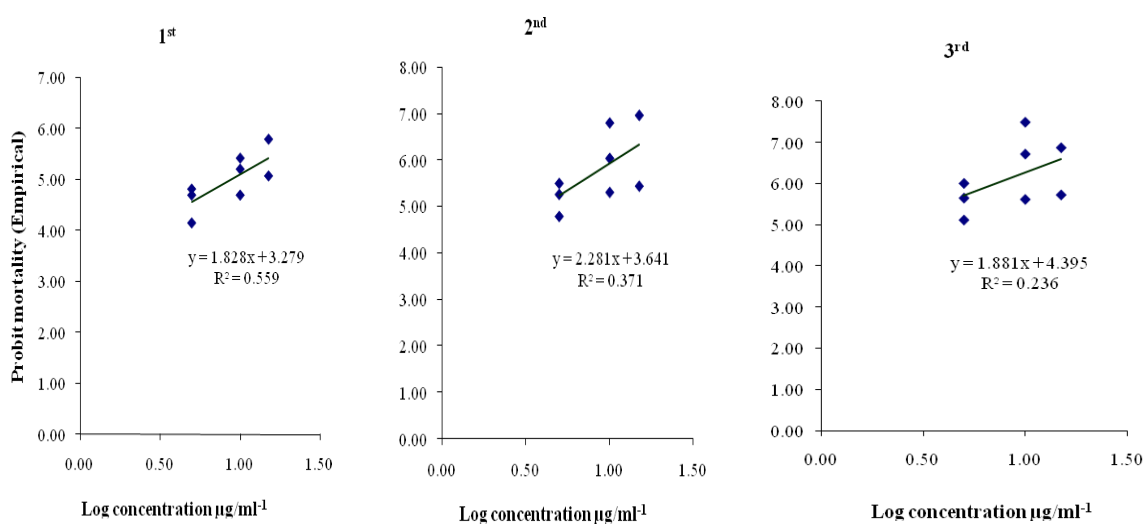

Figure 1.The log probit regression line for *A.indica* seed powder aqueous extract against sessile *A. tubercularis* (crawlers, adult females and males) 1st, 2nd & 3rd round treatment at Arjo Gudetu.

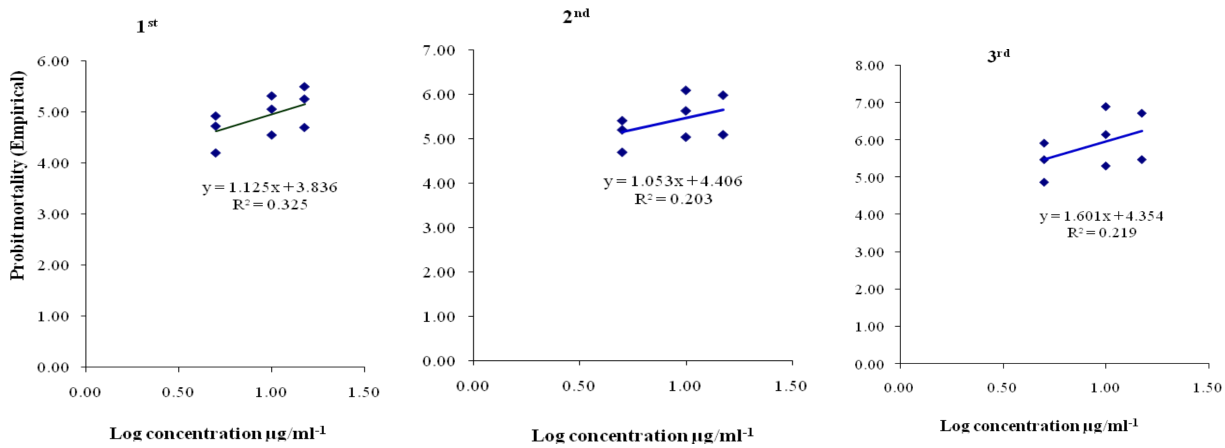

Figure 2.The log probit regression line for *A.indica* seed powder aqueous extract against sessile *A tubercularis* (crawlers, adult females and males) 1st, 2nd & 3rd round treatment at Uke.

DISCUSSIONS

There is little or no attempt to control *A. tubercularis* using *A. indica* water extracts. However, enormous research activities were done to utilize *A. indica* seed extracts in various forms including liquid form for the management of Atrhropod insect pests and mites. Debashri and Tamal (2012) mentioned that all parts of the *A. indica* tree posses' insecticidal activity but seed karnel is the most effective as it contains the highest concentration of the toxic chemical, Azadirtchin. Results of the current study showed that *A. indica* seed powder water extract was effective against *A. tubercularis*. The mortality percentage of *A. tubercularis* with the application of *A. indica* seed powder water extracts at Uke and Arjo Gudetu were relatively the same implying that there was no location variation.

The current finding agreed with the finding of Abdel-Aziz *et al*. (2016) who reported that the formulations of "Trilogy" (the trade name for neem oil) and other formulations against *A.tubercularis* was effective as it caused 81.03% mortality. Aziz *et al*. (2016) also added that "Trilogy" formulation caused 76.92% nymph mortality. At both experimental sites (Arjo Gudetu and Uke) the result of field spray with the *A.indica* seed powder water extracts were relatively the same. Significant difference was observed between male, female and crawlers of *A.tubercularis* in their susceptibility to *A.indica* seed powder water extracts implying that sex and developmental stage of the pest differently respond to the biopesticide tested. There was less percent reduction of adult females' population with *A. indica* seed powder water extracts at both sites. The less percent mortality of adult females *A. tubercularis* might be due to the hard exuvie, the fibrous impermeable wax like covering (scale) that covers the female body providing a protective barrier against physical and chemical damage (Foldi, 1990) and strongly attaching the scale to its host plant, adult females remain securely attached to the plant surfaces (leaves, stems, twigs and fruits) throughout their life.

The current study showed that the efficacy of neem seed derivatives at 15% concentration causes 83 to 86% mortality on the pre-adult stages of *A. tubercularis* which indicates that *A. indica* seed powder water extracts can be used by local people to control the infestation of *A. tubercularis* in anthropogenic habitats, especially in homestead areas.

The lethal concentration (LC_{50}) values of *A. indica* seed powder extracts at 10% and 15% concentrations against *A. tubercularis* showed good mortality at both experimental sites. The lethal concentration analysis indicated that regression coefficient had close to one in each case implying that the treatment made from *A. indica* seed powder aqueous water extracts was effective for management of *A. tubercularis.*

Based on the results of this study, the death percentages of *A. tubercularis* and the LC_{50} values of the *A. indica* seed powder water extracts essential constituents was determined. The results of this study indicated that the mortality of *A. tubercularis* increases with the increases in the concentrations of *A. indica* seed powder water extracts and number of frequencies of application. The results of this study indicated that the formulations from *A. indica* can replace commercial insecticides in IPM program. The current study agreed with the report of Chaudhary *et al*. (2017) who stated that Azadirachtin is one of the significant alternative strategies employing botanical pesticides, which is the most efficient means to replace the wide use of synthetic pesticides. This was supported Abdel-Aziz *et al*. (2016) who revealed that *A. indica* has an insecticidal effect for scale insect control and useful in reducing environmental pollution. Related study by Nahed *et al.* (2014) reported that *A. indica* compounds has an eco-friendly insecticidal effect on some scale insects, mealy-bugs, and its response to insecticidal effect on the population of *A. tuberularis (Aulacaspis mangiferae)*. They suggested the use of these essential oils as a potentially alternative source for developing bio-insecticides against scale insects.

A. indica is a potent botanical pesticide of choice for organic agriculture and it is widely used in several countries around the world today either singly in Integrated Pest Management (IPM) or in conjunction with Synthetic pesticides. There for data reported in this study shows that the 0.15 concentration of *A. indica* seed powder water extracts has better impact to knockdown the population of *A. tubercularis* and it can potentially be used for the management of the newly emerging and inflicting mango pest, *A. tubercularis*.

CONCLUSIONS

The insecticidal properties of natural plant products have been known since ancient times. Among the various plant products used as insecticides, the natural product formulations developed from neem (*A. indica*) have shown promise for pest management and it is absolutely non-toxic, biodegradable and environmentally friendly. Considering the high risks of chemical insecticides on human being, animals, and environment as well as the natural enemies, the botanical extract are a cheap, valuable, safe and environmentally friendly alternative insect pest management. In Ethiopia, considering the technologies towards *A. tubercularis* control, it is almost nil or little which enabled the pest to invade the whole country where mango is grown. Thus, the field experiment results suggested that *A. indica* seed powder water extract has a potential effect on sessile *A. tubercularis* under field conditions. Neem seed extract as botanical insecticide tested currently can be recommended against *A. tubercularis* management in the study areas and may be beyond.

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