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Effect of Three Plants Extracts on Some Bacterial Strains and *Culex pipiens* L. Stages

[1]A.A. Zaitoun, [2]M.H. Madkour and [2]M.Y. Shamy

[1]Department of Arid Land Agriculture Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdul-Aziz University, Saudi Arabia

[2]Department of Environmental Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdul-Aziz University, Saudi Arabia

Corresponding Author: A.A. Zaitoun, Department of Arid Land Agriculture Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdul-Aziz University, Saudi Arabia

ABSTRACT

Use of natural antimicrobial and insecticidal agents represents a safe vector control. Plant extract activity of *Calotropis procera*, *Acacia nilotica* and *Cassia senna* was investigated against some food borne pathogens (*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* sub sp. *aureus* and *Bacillus cereus*), food spoilage microorganisms (*Bacillus cereus* and *Bacillus stearothermophilus*) and towards larval mortality and development of *Culex pipiens* L. Antimicrobial activity of cold and hot aqueous extracts was studied. It was found that hot extract of *C. senna* has the strongest inhibition effect on *Staphylococcus aureus* sub sp. *aureus, Salmonella typhimurium* and *Bacillus stearothermophilus*. *Acacia nilotica* showed only complete inhibition of *Staphylococcus aureus* sub sp. *aureus, Salmonella typhimurium*. It was found that hot extracts of *Cassia senna* and *Acacia nilotica* have bactericidal effect on *Staphylococcus aureus* sub sp. *aureus* and *Salmonella typhimurium*. Acetonic plant extracts exhibited variable biological activity. The greatest was observed for *A. nilotica* which showed acute (2 days) and chronic (10 days) LC 50s of 212.1 and 144.2 ppm, respectively. Larval mortality up to 93.33% and reduction of egg hatchability was observed with *A. nilotica* extract. At every concentration level all plant extracts caused significantly high hindrance to subsequent larval development and reduced both pupation and adult emergence. Drastic retardation of development was shown by *A. nilotica* extracts when reared in very low concentration (100 ppm). However, *C. senna* and *C. procera* were more effective at higher concentrations. Application of such plant extracts to mosquito breeding sites may have great practical importance in relation to non-synthetic chemical control of these serious disease vectors.

Key words: Biological control, natural agents, disc assay methods, bactericidal activity, larval mortality

INTRODUCTION

It is known that many ornamental plant parts and extracts used as herbs and spices in foods are also known to possess antimicrobial activity (Huhtanen, 1980; Conner and Beuchat, 1984; Deans and Ritchie, 1987; Sunilson *et al*., 2009; Bele *et al*., 2009). Ethanolic extracts of 16 Turkish plant species were investigated for their antimicrobial activities against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris,*

Bacillus cereus, *Mycobacterium smegmatis*, *Listeria monocytogenes* and *Micrococcus luteus*. Each plant species has activity against different microorganisms (Dulger and Gonuz, 2004).

Singh *et al.* (2005) reported that herbs and spices are among the most important targets to search for natural antioxidants and antimicrobial agents. For example clove oil was found to be very effective against *Staphylococcus epidermidis* and *Staphylococcus* sp. (Joseph and Sujatha, 2011). Also, *Cinnamomum verum* stem bark aqueous extract represents antimicrobial activity against some food-borne pathogen bacteria (Puangpronpitag and Sittiwet, 2009).

Growth of food spoilage molds, yeasts and bacteria is reduced in the presence of many herbs and herbs commonly used as flavoring agents (Beuchat and Golden, 1989). Also, bacteria of public health significance are known to be adversely affected by certain compounds present in these seasoning agents include *Clostridium botulinum*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Vibrio parahaemolyticus* and *Escherichia coli*.

Tayel and El-Tras (2009) investigated the antimicrobial activity of twenty five herbs and spices used in folk medicine by Egyptian housewives to treat gastrointestinal disorders against seven bacterial strains, mostly food borne including pathogens. They found that herbs and spices extracts could be successfully applied as natural antimicrobials for elimination of food borne bacteria and pathogens growth.

Insect-transmitted diseases remain a major source of illness and death worldwide. Mosquitoes alone transmit diseases in more than 700 million people annually (Taubes, 1997). These diseases including malaria, filariasis, yellow fever, dengue and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries (Jang *et al.*, 2002; Rajkumar and Jebanesan, 2005). Control of such diseases is becoming increasingly difficult because the over production of detoxifying mechanisms of chemical insecticides has reported for *Culex* species (Severini *et al.*, 1993). On the other hand, majority of mosquito species have developed high levels of resistance to microbial control agents (Rao *et al.*, 1995). One alternative approach is the use of natural insecticides from plant origin (El-Hag *et al.*, 1996; Raghavendra *et al.*, 2011). The botanical insecticides are generally pest specific and are relatively harmless to the non-target organisms including man. They are also biodegradable and harmless to environment. One plant species may possess substances with a wide range of activities, like extracts from *Azadriachta indica* which showed antifeedent, antioviposition, repellent and growth regulating activity (Schmutterrer, 1995). Insecticidal activity of many plants against several insect pests has been demonstrated (Carlini and Grossi-de-Sa, 2002; Kundu *et al.*, 2007; Ohaga *et al.*, 2007; Boussaada *et al.*, 2008; Chakkaravarthy *et al.*, 2011). The three plants of *Calotropis procera*, *Acacia nilotica* and *Cassia senna* are available in many parts of Saudi Arabia and used in folk medicine (Migahid, 1978).

The aim of present study was to investigate the antibacterial and insecticidal effects of some plant extracts on some bacterial strains and insect larvae. Also, was to examine effect of these extracts on mosquito egg hatchability.

MATERIALS AND METHODS

This study was conducted in the year 2010.

Organisms:

- *Escherichia coli* DSM 5212
- *Salmonella typhimurium* DSM 5569

- *Staphylococcus aureus* sub sp. *aureus* DSM 20231
- *Bacillus cereus* DSM 2302
- *Bacillus stearothermophilus* DSM 297

The aforementioned bacterial strains were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. These strains were subculture on nutrient agar and stored at 4°C. All cultivations of the strains of *E. coli*, *Salmonella typhimurium*, *B. cereus* and *aureus* sub sp. *aureus* were at 37°C for 24 h and at 55°C for 48 h for *B. stearothermophilus* using nutrient broth.

Insects: Insects and second instar larvae were obtained from culture of *Culex*, reared at laboratory, maintained on pigeon blood and 10% sucrose solution. The experiment was carried out at the Faculty of Meteorology, Environment and Arid land Agriculture, King Abdul-Aziz University, Jeddah, Saudi Arabia. Larvae were reared in tap water.

Plant aqueous extracts: The cold extraction was done at room temperature after milling the tested material by mixing them with distilled water at room temperature to give high concentrations. The concentrate extract was then sterilized by filtration (Sartorious membrane filters, 0.2 μm, Germany). The sterilized extracts were diluted by the growth medium to advise level. Placing a certain weight of herbs in cloth bags did the hot extraction. The weight of herbs was selected to give the required expected concentrations. The bags containing herbs were submerged in the nutrient both medium (or distilled water in case of agar diffusion procedure) and autoclaved at 121°C for 15 min. The test of kill curve was done in these previous medium where hot and cold herbs extracts were placed.

Plant acetonic extracts: Plant materials of *Calotropis procera*, *Acacia nilotica* and *Cassia senna* were collected from different parts in Saudi Arabia. *Calotropis procera*, *Cassia senna* leaves and *Acacia nilotica* seeds were air- dried for 48 h, ground to fine parts and extracted with acetone at ambient temperature. A gentle warming to 35-40°C was sometimes found necessary. The mixture was stirred for 30 min by magnetic stirrer and left 24 h. Then, it was condensed in a vacuum rotary evaporator from solvent in a water bath at 55°C according to Severini *et al.* (1993). The extracts were then freeze dried using a Labconco Freeze Dryer-18 model 75018 for 48-72 h. Stock solution was prepared from the lyophilized residue.

Antibacterial activity

Agar diffusion: Agar diffusion has been conducted by using the method of Davidson and Parish, (1989). It has often been referred as the disc assay. The antimicrobial compound is applied to agar plate, using an impregnated filter paper disk. The study discs were sterilized separately and impregnated under sterile condition when cold extraction was done at room temperature. However, the filter paper discs were impregnated and sterilized during the hot extraction at 121°C/15 min.

Inhibition or kill curve: This test demonstrated by Schoenknecht *et al.* (1995) involves inoculation of one of the tested bacteria into the liquid medium, addition of one of the tested herbs, followed by incubation and periodic sampling to determine growth or survival.

Test procedure towards insects: Stock solutions of the three plant acetonic extracts were prepared by dissolving the extracted powder in warm distilled water (at 0.5 g/100 mL water). Different concentrations of 100, 200, 300, 400 and 500 ppm were prepared from stock solution. Twenty freshly laid eggs or ten second instar larvae were transferred from the culture into plastic cups (8 cm diameter, 10 cm deep), each containing 30 mL of desired concentration. Treatments were triplicated and control had only distilled water. Larvae were fed *ad libitum* and kept under laboratory conditions. Egg hatchability was determined at 4 and 7 days after treatment. Larval mortalities were counted at 2, 4 and 10 days after treatment. Percentage of successful pupation and adult emergence were determined by monitoring on daily basis until all adults in the control have emerged.

Statistical analysis: Data were analyzed using maximum likelihood procedures and values of LC_{50} were calculated according to Finney (1971). Data were corrected for control mortality (Abbott, 1925). Data of egg hatchability were analyzed by analysis of variance. If significant differences ($p<0.05$) occurred, means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Table 1 shows the inhibitory or bactericidal effect of *Calotropis procera*, *Acacia nilotica* and *Cassia senna* using the disc assay. Different concentrations of tested materials were used (1, 5 and 10% as aqueous extracts). The results of the 1% concentration were presented in Table 1. Samples with no inhibitory effect in Table 1, did not show any further response when their concentration were raised. It can be seen that hot extraction give higher inhibition when compared with the cold extraction. Senna has completely inhibited *Staphylococcus aureus* sub sp. *aureus*, *Salmonella typhimurium* and *Bacillus stearothermophilus*. This is in agreement with Liu and Nakano (1996). This is in contradiction with Holzminden (1982) who found that *Salmonella* strains were less inhibited than *Staphylococcus* strains.

Figure 1 shows the effect of *Cassia senna* and *Acacia nilotica* on *Staphylococcus aureus* sub sp. *aureus*. It is clear that *Acacia nilotica* has bactericidal effect. Bactericidal effect was achieved after 8 and 18 h incubation for *Acacia nilotica* and *Cassia senna*, respectively.

Figure 2 illustrates the effect of *Cassia senna* and *Acacia nilotica* also on *Salmonella typhimurium*. Both of them have bactericidal effect after incubation time of 8 and 12 h for *Cassia senna* and *Acacia nilotica*, respectively. It is clear that *Cassia senna* has more bactericidal activity

Table 1: Antibacterial effect of some herbs extracts (hot and cold extraction) at 1% concentration on some food born pathogens and spoilage microorganisms nsing disc assay

	Microorganisms				
Type of herbs	*E. coli*	*Staph. aureus* sub sp. *aureus*	*Salmonella typhimurium*	*B. cereus*	*B. stearothermophilus*
Cassia senna (1)	-	+++	+++	+	+++
Cassia senna (2)	-	+	+	-	+
Calotropis procera (1)	+	-	-	-	±
Calotropis procera (2)	-	-	-	-	-
Acacia nilotica (1)	+	+++	+++	-	-
Acacia nilotica (2)	-	+	+	-	-

1: Hot extraction, -: No inhibition, ++: Slight inhibition; 2: Cold extraction, +: Very slight inhibition and +++: Complete inhibition

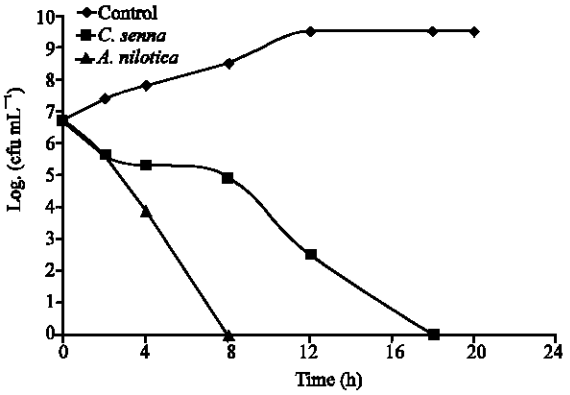

Fig. 1: Effect of *C. senna* and *A. nilotica* on *Staphylococcus aureus* sub sp. *aureus*

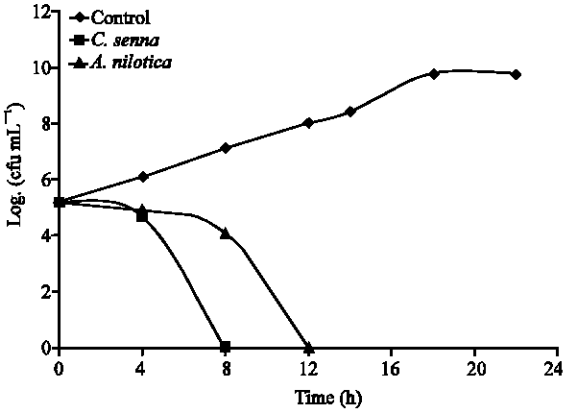

Fig. 2: Effect of *C. senna* and *A. nilotica* sage on *Salmonella typhimurium*

against *Salmonella typhimurium* (G^-) than *Staphylococcus* (G^+). This is in contradiction with (Imelouane *et al.*, 2009).

Figure 3 shows that senna has bactericidal effect on *Bacillus stearothermophilus* after incubation time of 25 h. From Fig. 1-3, it clear that *Cassia senna* has more bactericidal activity against *Salmonella typhimurium* (G^-) than *Staphylococcus aureus* sub sp. *aureus* and *Bacillus stearothermophilus* (G^+). This is in contradiction with Imelouane *et al.* (2009).

The mortality (%) of *C. pipiens* larvae treated with three plant extracts in acetone and their LC_{50} values and 95% confidence limits at 2, 4 and 10 days after treatment are shown in Table 2 and 3. The mortality (%) of *C. pipiens* larvae treated with plant extracts in acetone is given in Table 2. Data showed that larvae suffered up to 93.0 and 82.8% mortality after 10 days of exposure to 500 ppm *A. nilotica* and *C. senna* extracts, respectively. However, the lowest *A. nilotica* concentration caused 45% mortality after 2 days of treatment. *C. procera* extracts caused the lowest mortalities, while highest concentration (500 ppm) caused 70.7% mortality after 10 days of treatment. LC_{50s} and 95% Confidence Limits (CL) for each plant are given in Table 3. Data showed a significant differences. Acute and chronic LC_{50} (2 and 10 day of mortality) for second instar larvae were 212.1 and 144.2, 301.5 and 173.1 and 466.7 and 251.7 for *A. nilotica*, *C. senna* and C. *procera*, respectively. *A. nilotica* was significantly more toxic at all exposure times than *C. senna* and *C. procera*.

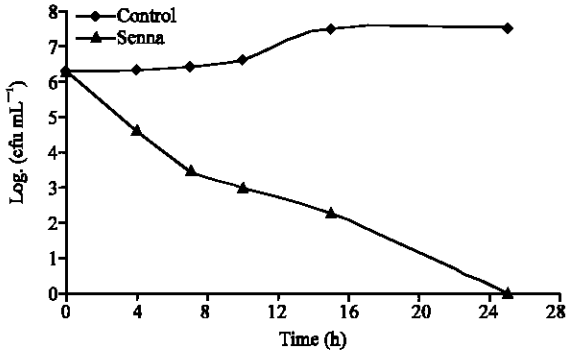

Fig. 3: Effect of *C. senna* on *Bacillus stearothermophilus*

Table 2: Mortality percentage of *Culex pipiens* larvae reared in media containing acetonic plant extracts

		Mortality (%)		
Plant extract	Conc. (ppm)	2 days	4 days	10 days
Acacia nilotica	100	45.00	56.72	66.76
	200	50.00	63.31	76.76
	300	53.31	66.76	83.31
	400	58.33	78.76	90.00
	500	68.34	90.00	93.00
	Control	00.00	00.00	03.33
Cassia senna	100	22.60	32.20	61.00
	200	35.90	48.20	70.70
	300	43.10	61.00	73.30
	400	50.00	71.34	76.70
	500	61.00	76.70	82.80
	Control	00.00	6.70	00.00
Calotropis procera	100	8.20	12.50	18.60
	200	17.30	26.30	46.60
	300	27.80	42.60	61.11
	400	44.44	56.67	66.67
	500	50.00	64.44	72.22
	Control	03.00	00.00	00.00

Table 3: LC_{50} values and 95% confidence limits for *Culex pipiens* larvae in media containing acetonic plant extracts

Plant extract	Assay time (days)	Slope	LC_{50} (95% CL)
Acacia nilotica	2	0.97	212.1 (140.8-433.5)
	4	1.20	195.5 (131.6-405.3)
	10	1.46	144.2 (72.7-310.4)
Cassia senna	2	0.76	301.5 (195.6-467.9)
	4	1.35	235.6 (131.3-399.9)
	10	1.44	173.1 (101.2-324.5)
Calotropis procera	2	1.03	466.7 (305.9-600.1)
	4	1.76	305.5 (190.3-465.6)
	10	1.86	251.7 (141.3-380.2)

Egg hatchablity was significantly lower ($p<0.05$) in all extracts than control (Table 4). At 100 ppm concentration, *A. nilotica* had the most severe effect on egg hatching rate which was

Table 4: Egg hatchability percentage of *Culex* in media containing acetonic plant extracts

Plant extract	Conc. (ppm)	Egg hatchability (%)
Acacia nilotica	100	69.2cd
	200	60.0de
	300	49.3ef
	400	32.5g
	500	21.4h
	Control	98.0a
Cassia senna	100	81.7bc
	200	75.5c
	300	67.6cd
	400	50.0ef
	500	43.6ef
	Control	98.2a
Calotropis procera	100	86.7b
	200	81.7bc
	300	75.5cd
	400	70.3cd
	500	64.0d
	Control	98.2a

Means followed by the same letter are not significantly different at 5% level, Duncan multiple test

Table 5: Successful pupation and adult emergence of *Culex pipiens* larvae reared in media containing acetonic plant extracts

Plant extract	Conc. (ppm)	% successful pupation	% successful adult emergeuce pupation adult emergence
Acacia nilotica	100	20.30	9.40
	200	16.10	8.11
	300	10.12	4.60
	400	5.90	1.78
	500	00.00	00.00
	Control	99.40	98.00
Cassia senna	100	39.30	14.78
	200	22.43	9.32
	300	14.89	6.60
	400	8.41	2.41
	500	1.21	00.00
	Control	100.00	100.00
Calotropis procera	100	68.10	40.00
	200	58.10	20.20
	300	50.00	19.60
	400	31.40	9.05
	500	10.00	4.53
	Control	96.60	93.20

reduced by about 30% at their highest concentration (500 ppm). The three plant extracts reduced egg hatchability by about 79, 57 and 36%, for *A. nilotica*, *C. senna* and *C. procera*, respectively.

The effect of the three plant materials on growth and development of *C. pipiens*, larvae to adulthood are given in Table 5. There was considerable reduction in the percentage of larvae undergoing successful pupation in all treatments compared with control. No further larval development took place beyond the second instar in the *A. nilotica* 500 ppm concentration. On the

other hand all plant extracts had an evident inhibitory effect even at their lowest concentrations (100 ppm) where the successful pupation were only 20.3, 39.3 and 68.1 for *A. nilotica*, *C. senna* and C. *procera*, respectively. Complete suppression of adult emergence was evident in the 500 ppm concentration of *A. nilotica* and *C. senna*. The adult emerging percentages from the 100 ppm treatments were 9.40, 14.78 and 40.0% for *A. nilotica*, *C. senna* and C. *procera*, respectively, compared with control.

Considerable biological activity related to toxicity and hindrance of growth and development of the larvae of *C. pipiens* has been observed in this study. From the three plant extracts, *A. nilotica* was found to cause higher rate of mortality compared to other plant extracts. In agreement with the present study, the activity of *A. nilotica* seeds extracts has been attributed in part to the saponins and alkaloids in the seeds (Duru and Onyedineke, 2010). *C. senna* and *C. procera* exhibited a relatively mild acute effect on mosquito larvae especially in its lower concentrations. However, its chronic toxicity was almost high above 200 ppm.

CONCLUSION

The *Cassia senna* has faster bactericidal activity against *Salmonella typhimurium* than *Staphylococcus aureus* sub sp. *aureus* and *Bacillus stearothermophilus*. The extracted plant materials specially *A. nilotica* and *C. senna* have toxic, growth and development-retarding influence on *C. pipiens* mosquitoes. Moreover, application of these materials is not likely to leave harmful residues in the environment since they are naturally occurring among the local flora.

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