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Antibiotic Effects of *Argemone mexicana* (*Papaveraceae*) against Field Crops and Pathogens Causing Mastitis in Dairy Cattle in three Districts of Amhara Region, Ethiopia

Kefyalew Alemayehu[1*] and Tadesse Desalegn[1]

[1]Biotechnology Research Institute, Bahir Dar University, P.O.Box 21 45, Bahir Dar, Ethiopia.

Authors' contributions

This work was carried out in collaboration between both authors. Authors KA and TD designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KA and TD managed the analyses of the study. Authors KA and TD managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The destructive nature of *Argemone mexicana* against field crops and grazing lands as invasive species and, the antibiotic effect for treating common diseases of dairy cattle causing mastitis were investigated at Farta, Gondar and Bahir Dar zuria districts. Plant materials were extracted and the bacteria were isolated from milk of cows infected by mastitis. Finally, sensitivity tests were conducted using standard laboratory procedures. The results revealed that the mean distribution of *Argemone* in roadside land use type (18.53±6.2) was higher than that of fallow land (4.25±0.75) and farmland (2.72±0.44). The mean abundance per transect belt in 2013 in Farta district (8.5±8.3) was higher than that of Bahir Dar zuria (7.7±8.0) and Gondar district (5.6±4.4). The antibacterial activity of oil cakes extracted from cooked and raw seeds, and fresh plant juice extracts revealed strong inhibition in bacterial growth. Cakes produced from cooked *Argemone* seeds shown strong inhibitory effect on *Escherichia coli* than *Staphylococcus aureus,* and *Streptococcus aglaciatae*. As the distribution and abundance of the plant increased, the abundance and distribution of native

**Corresponding author: E-mail: kefyale@gmail.com;*

plant species decreased. However, *Argemone* plant extracts shown excellent potential in treating diseases of mastitis.

Keywords: Argemone; antibiotic; dairy cattle; mastitis; prevalence.

1. INTRODUCTION

The rapid increase in invasive species is homogenizing the world's flora and fauna and is recognized as a primary cause of global biodiversity loss [1]. Biological invasion is considered as a form of biological pollution and one of the major causes of species extinction [2] Alien plants can spread rapidly due to the intentional transportation of ornamental and forage plants [3]. However, some plants may be rich source of antimicrobial agents and are used as source of many dominant and power full drugs [4].

The genetic ability of pathogenic bacteria to develop resistance against commonly used antibiotics is a major medicinal problem and challenge worldwide, posing a big threat to human society [5]. On a global basis, at least 130 drugs, all single chemical entities extracted from plants, or modified further synthetically are currently in use [6]. A number of plant secondary metabolites like alkaloids and flavonoids have been used as antiviral, antibacterial, antiamoebal and anticancer agents [7]. Almost any bacterial or *Mycoplasma* spp that can opportunistically invade tissue and infect can cause mastitis which infects dairy cows with poor management. However, most infections are caused by various species of streptococci, staphylococci, and Gram-negative rods, especially lactose-fermenting organisms of enteric origin, commonly termed coliforms. From an epidemiologic standpoint, the source of infection may be regarded as contagious or environmental. However, except for *Mycoplasma* spp which may spread from cow to cow through aerosol transmission and invade the udder subsequent to bacteremia, contagious pathogens are spread during milking by milkers' hands or the liners of the milking unit. Species that utilize such mode of transmission include *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli.* These bacteria have developed multiunit drug resistance in recent years. This can be witnessed by the ever increasing reports of drug resistance problems from various corners.

Argemone mexicana is a 50 cm tall herb of poppy species, having pale to bright yellow flower and black brown seed [8]. It is a member of family *Papaveraceae,* endemic to Mexico and now widely naturalized in the United States, India and Ethiopia [8]. As a weed, it is commonly found along with pulses, cereals, tobacco, tea, and sugarcane, tomatoes, cotton and potato in Ethiopia. The mean prevalence of *Argemone* plants in roadside land use type is higher than that of fallow land and farmland in Ethiopia [8]. The number of pods produced per a single plant and number of seeds produced per single pod is too much to create devastating effects to native plant species when germinate. The overall increase in areas where the prevalence and distribution *Argemone* studied was 14.95% from 2006 -2010. *Argemone* cake is also toxic for animal feeds [9]. However, no adequate information was about collected antibiotic effects of *Argemone mexican* (*Papaveraceae)* against field crops and pathogens causing mastitis in dairy cattle, in Amhara Region, Ethiopia.

Therefore, the objectives of the study was to investigate the destructive nature of *A. mexicana* against field crops and grazing lands as invasive species and, to investigate the antibiotic effect of *A. mexicana* against *S. aureus*, *S. agalactiae*, and *E. coli* causing mastitis in dairy cattle"

2. MATERIALS AND METHODS

2.1 Study Area Descriptions

The study was conducted at three districts of Amhara National Regional State (ANRS) namely, Farta, Gondar zuria and Bahir Dar zuria districts in 2013. The Farta district is found South Gonder zone, Amhara National Regional State (ANRS). The mean annual rainfall is 1651 millimetres (mm). The mean monthly average temperature is 18.4°C. The altitudes range from 1500 - 4135 meters above sea level. Gondar zuria district is found in the North Gonder zone. The altitude ranges from 1966 - 2133 meters above sea level. The mean annual rainfall is 1161 mm. The average temperature is 19.1°C. The third study area, Bahir Dar zuria district, is found in West gojjam zone. The altitude ranges from 1786 - 1969 meters above sea level. Mean annual rainfall of 1224 mm and the mean annual daily

temperature recorded is 18.5°C. The main crops produced in these three study areas are barley, wheat, teff, and other pulse crops. The average length of growing period of the plants in all study sites ranges from 120 -270 days.

2.2 Data Types and Collection Methods

Data were collected using desk reviews and secondary data collection, participatory rural appraisal, field observation with direct measurements and laboratory analysis for investigating antibiotic effects.

2.2.1 Desk reviews and secondary data collection

Routine activities and reporting formats were developed and thoroughly assessed at different pertinent offices. Based on the assessments of secondary data from literature and desk reviews with in pertinent organization were conducted.

2.2.2 Focus group discussion

The focus group discussions were formed by selecting communities of the targeted population in each district. Two focus group discussions were conducted at each selected peasant associations (PA). Therefore, a total of 12 focus group discussions were conducted during the study period. Each focus group discussion was composed of about six discussants.

2.2.3 Field observation and direct measurements

During the study, field visits and observation to the study areas were conducted. Identification of land use types, determination of sampling units, sample collection, and identification were carried out. The study sites within the study area were purposely selected to see the prevalence per land use types and stratified into three different land use types with 25 belts transect each as roadside, fallow land and farmland following. A total of 225 transect belts were used (25 transect belts × 3 study areas × 3 land use types). The size of each belt transect were 100 meters by 5 meters. The data collection for seeds was carried in the month of October and May of the study year which were the appropriate period for seed [10] Following plant abundance and distribution, number of pods per plant, number of seeds per pod and number of seeds germinated per pod were estimated.

2.3 Methods of Investigating Antibiotic Effects through Laboratory Analyses

2.3.1 Plant materials extraction

Fresh *Argemone* seeds were collected from these sampled plants in the study area and washed thrice with distilled water and were dried using blotting paper in the laboratory. Subsequently, seeds were grinded using mortar and pestle (seed grinding machines). The seed was grinded as raw and cooked before oil and cake extraction. Oil extraction from raw and cooked *Argemone* cake was conducted using maceration technique following [11]. Fresh plant juice sap was also taken during laboratory analyses day.

2.3.2 Isolation of mastitis causing bacteria from milk samples

Milk samples were collected from cows infected with mastitis and tested using CMT procedures (California mastitis test). The organisms employed for the trial are three known bacteria to have caused mastitis in many causes. Samples which showed gel formation (positive result) to this test were considered for further bacteriological analyses. Milk samples were also collected aseptically in accordance to the national mastitis council. The bacteria *S. aureus*, *S. agalactiae*, and *E. coli* were isolated from cows diagnosed with mastitis, in Amhara regional animal health Lab. Bacteriological examinations were conducted. A loop full of the sample was inoculated on to blood agar base enriched with 5% sheep blood and MacConkey agar by applying the quadrant streaking method or respective quadrant. The plates were incubated aerobically at 37°C for 24-48 hrs. Following this, gross colony morphology coloration and haemolytic potentials were thoroughly examined and characterized after the stated time of incubation.

2.3.3 Sensitivity test

This was done on diagnostic sensitivity test media using Mueller-Hinton agar media. The bacterial growth of 3-4 colonies were picked up from the surface of 24 hrs culture and suspended in 5 ml tryptic soy broth by adjusting to a turbidity equivalent to 0.5 MacFarland Turbidity standards. The adjusted inoculums were swabbed on Mueller-Hinton agar media and kept under 37°C in an incubator to determine the susceptibility of the bacteria under consideration

to the different extracts of the plant. These extracts were applied directly on the agar media as raw powder, oil, heat treated powder and heat treated oil as well as fresh plant extract (juice). The fresh plant extract squeezed to directly apply the sap on the swabbed agar surface immediately after cutting the stem of the plant by sterile scissor.

2.4 Data Management and Analyses

The survey data was analysed using SAS, Version 9.1.3 (2008) and the mean distribution of the different variables were analysed using descriptive statistics and the differences in distribution among the districts were analysed using ANOVA. Data on the response of test organisms to the extracts was compiled through observational method and presented in the form of graphs and tables.

3. RESULTS

3.1 Focused Group Discussions

One hundred and twenty (120) small holder farmers (45 in Farta, 36 in Gondar zuria and 39 in Bahir Dar zuria) were interviewed and involved in focus group discussions. From the group discussion it was possible to understand that the distribution of the plant is increasing from time to time. The farm lands and the grazing lands are highly invaded by this plant. The discussants also stressed that the plant and its product is useless for the farmers except that some females use it for oiling during backing of loaf (Ethiopian enjera).

3.2 Distribution per Land Use Type

From the statistical analyses it was possible to see that the mean distribution of *A. mexicana* in roadside land use type (18.53±6.2) was higher than that of fallow land (4.25±0.75) and farmland (2.72±0.44). The number of pods produced per a single plant and number of seeds produced per a single pod were too much to create devastating effects to native plant species when germinate. The abundance (quantity) and prevalence of native plant species under the shed of *A. mexicana* in road side, fallow land and farm land per transect belt at Farta district were too low and decreasing from time to time (1.13±1.2, 1.8±2.5 and 1.08±0.6) as compared to the abundance and distribution of *A. mexicana.* The mean abundance of *Argemone* plant per transect belt in 2013 in Farta district (8.5±8.3) was higher than that of Bahir Dar zuria (7.7±8.0) and Gondar district (5.6±4.4) (Table 1 and Fig. 1).

The mean numbers (mean ± STD) seeds produced per pod in Farta, Gondar zuria and Bahir dar zuria districts were 425.75±107.15, 338±21, 357±95, respectively (Table 2 and Fig. 4).

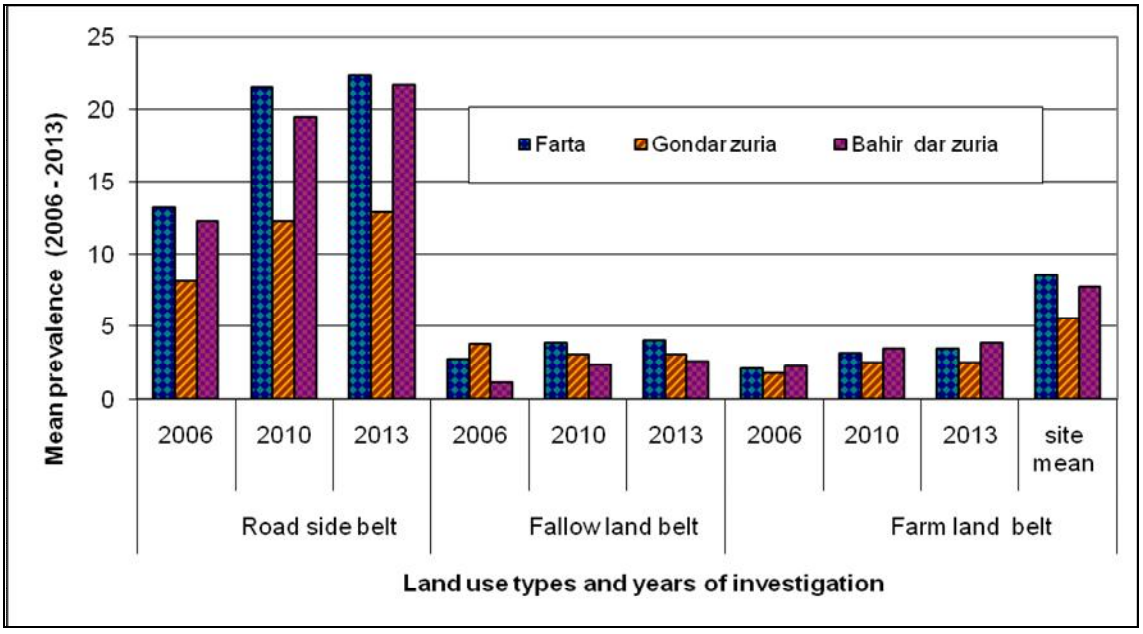

Fig. 1. Intensity and prevalence of *A. mexicana* per land use types

Table 1. Number of *Argemone* plants in 2006, 2010 and 2013 in the study districts

	Land use types mean (Site Mean±STD: Farta: 8.5±8.3, Gondar : 5.6±4.4, Bahir Dar: 7.7±8.0)											
	Road side belt (100 m × 5 m)				Fallow land belt (100 m × 5 m)				Farm land belt (100 m × 5 m)			
	2006	2010	2013	Mean±STD	2006	2010	2013	Mean±STD	2006	2010	2013	Mean±STD
Farta	13.22	21.6	22.4	19.07±5	2.8	3.9	4.02	3.6±0.7	2.1	3.2	3.53	2.9±0.8
Gondar	8.1	12.3	12.9	11.1±3	3.8	3.1	3.11	3.4±0.4	1.8	2.5	2.56	2.3±0.5
Bahir Dar	12.3	19.5	21.7	17.8±4.9	1.2	2.4	2.62	2.1±0.8	2.3	3.5	3.92	3.2±0.8

Table 2. The mean number of seeds produced per pod in the study districts

Districts	N obs	Variables	Mean	Std dev	Std error	Variance	t -value	Pr > |t|
Farta	100	Main branch	2.22	0.12	0.04	0.02	169.68	<.0001
		Sub-branch	6.76	0.51	0.05	0.34	115.87	<.0001
		Total branch	15.72	1.66	1.66	2.75	94.80	<.0001
		Number of pods per branch	23	1.72	1.72	2.97	38.30	<.0001
		Number of seeds per plant	374	14636.67	1463.67	214232046	14.06	<.0001
		Number of seeds per pod	425.75	117.15	10.75	11181.30	39.73	<.0001
		Total pods per plant	19.38	4.12	1.41	98.99	13.74	<.0001
		Total seed per plant	7915.63	5922.84	592.21	15079997.16	13.40	<.0001
Gondar Zuria	100	Main branch	1.65	0.24	0.02	0.06	60.67	<.0001
		Sub-branch	4.47	0.33	0.03	0.12	125.83	<.0001
		Total branch	7.32	1.11	0.11	1.24	65.91	<.0001
		Number of pods per branch	22.9	1.73	0.18	3.17	10.09	<.0001
		Number of seeds per plant	373.97	13331.09	1333.10	135110315	15.62	<.0001
		Number of seeds per pod	333.21	110.62	10.01	10124.13	33.61	<.0001
		Total Pods per plant	25.76	2.16	0.26	4.69	118.99	<.0001
		Total seed per plant	8718.59	2706.17	273.62	7408242.51	31.90	<.0001
Bahir Dar Zuria	102	Main branch	1.41	0.31	0.02	0.10		
		Sub-branch	3.71	0.63	0.01	0.16	15.84	<.0001
		Total branch	5.28	1.45	0.14	2.10	55.69	<.0001
		Number of pods per branch	7.32	3.25	0.32	10.54	36.75	<.0001
		Number of seeds per plant	64211.79	15190.16	1522.86	236549361	24.65	<.0001
		Number of seeds per pod	357.95	10.25	8.94	8144.98	12.21	<.0001
		Total pods per plant	25.10	1.05	0.11	1.11	10.06	<.0001
		Total seed per plant	2919.11	2114.71	229.19	5357898.17	211.17	<.0001

3.3 Sensitivity Test

The analyses of maximum likelihood estimates of SAS procedures indicated that the main hazard in the mean distribution and prevalence per land use types was from the germination of seeds. This is because high hazard ratio was recorded from the main branch distribution of the plant. However, significant amounts of oil and cake were extracted from the plant for selectivity test analyses against bacteria causing mastitis. The antibacterial activity of oil cakes extracted from cooked *Argemone* seed revealed strong reduction in bacterial growth in terms of zone of inhibition. The zone of inhibition was increased with increased in number of days for the all bacterial species. Cakes produced from cooked *Argemone* seed possessed greater inhibitory effect on *E. coli* than *S. aureus* and *S. agalactiae* (Fig. 2.)

On the other hand, the application of fresh plant juice extracted from *A. mexicana* for the treatment revealed stronger antibacterial activity against *S. aureus than E. coli and S. agalactiae* (Fig. 3).

In almost all test organisms, the application of oil extracted both from cooked and raw *Argemone* seeds, no visible inhibition was recorded. This might be attributed to the viscous nature of the oil that it didn't manage to diffuse in to the agar medium as best it can.

The application of cakes extracted from raw *Argemone* seeds had roasting smell during crashing and high fungal growth was observed for both cases.

4. DISCUSSION

The mean prevalence of *Argemone* plants in roadside land use type (17.41±5.9) was higher than that of fallow land (3.35±0.8) and farmland (2.65±0.8) in Farta district in 2006 and 2010 [10]. But in 2013, the mean distribution of *A. mexicana* in roadside land use type (18.53±6.2) was higher than that of fallow land (4.25±0.75) and farmland (2.72±0.44) and shown increasing trend. The number of pods produced per a single plant and number of seeds produced per a single pod were too much to create devastating effects to native plant species when germinate even higher than in 2006 and 2010.

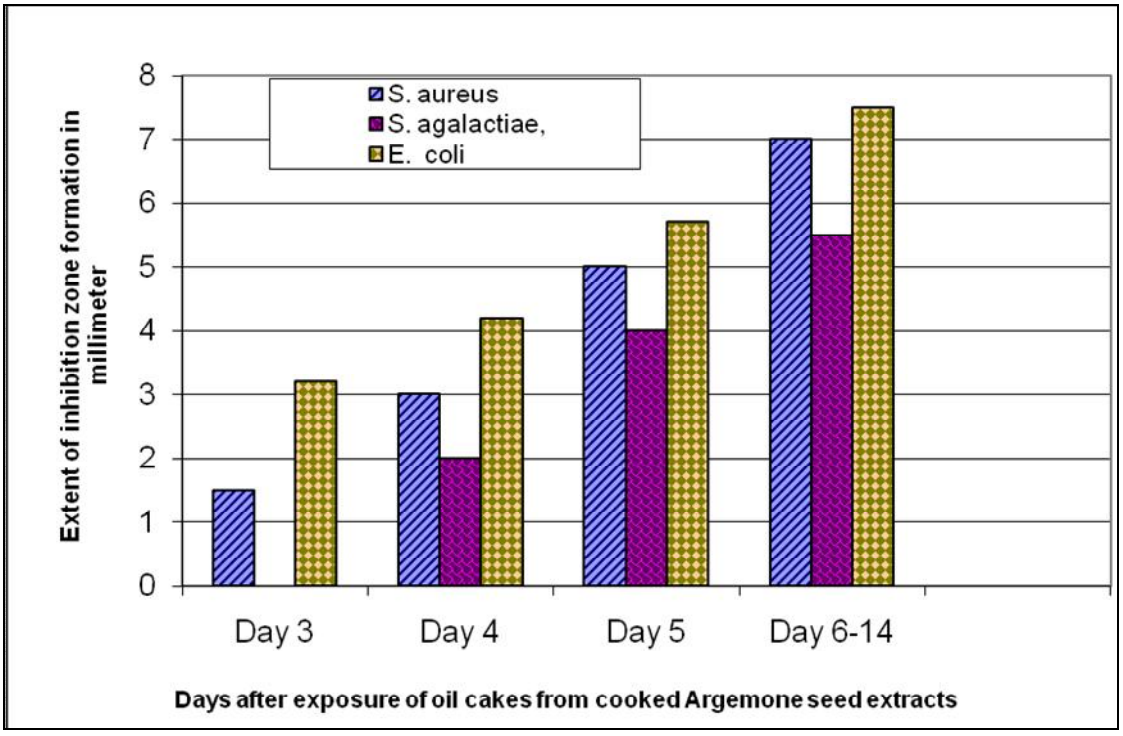

Fig. 2. Inhibition effect of cooked *Argemone* seed against *E. coli*, *S. aureus* and *S. agalactiae*

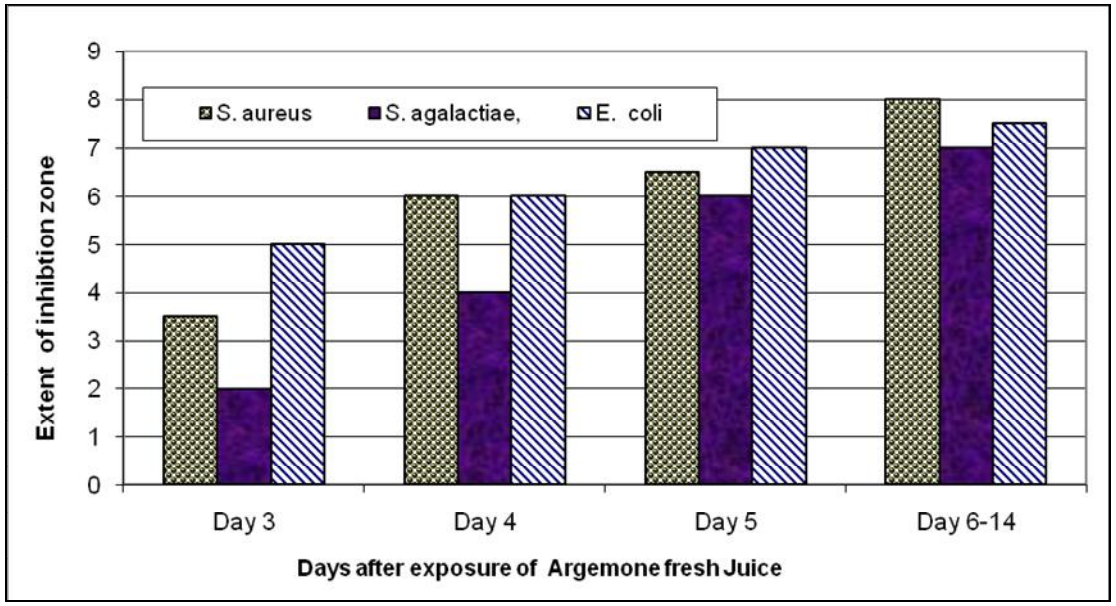

Fig. 3. Inhibition effect of fresh plant juice extracts of *A. exicana* against *S. aureus, E. coli and S. agalactiae*

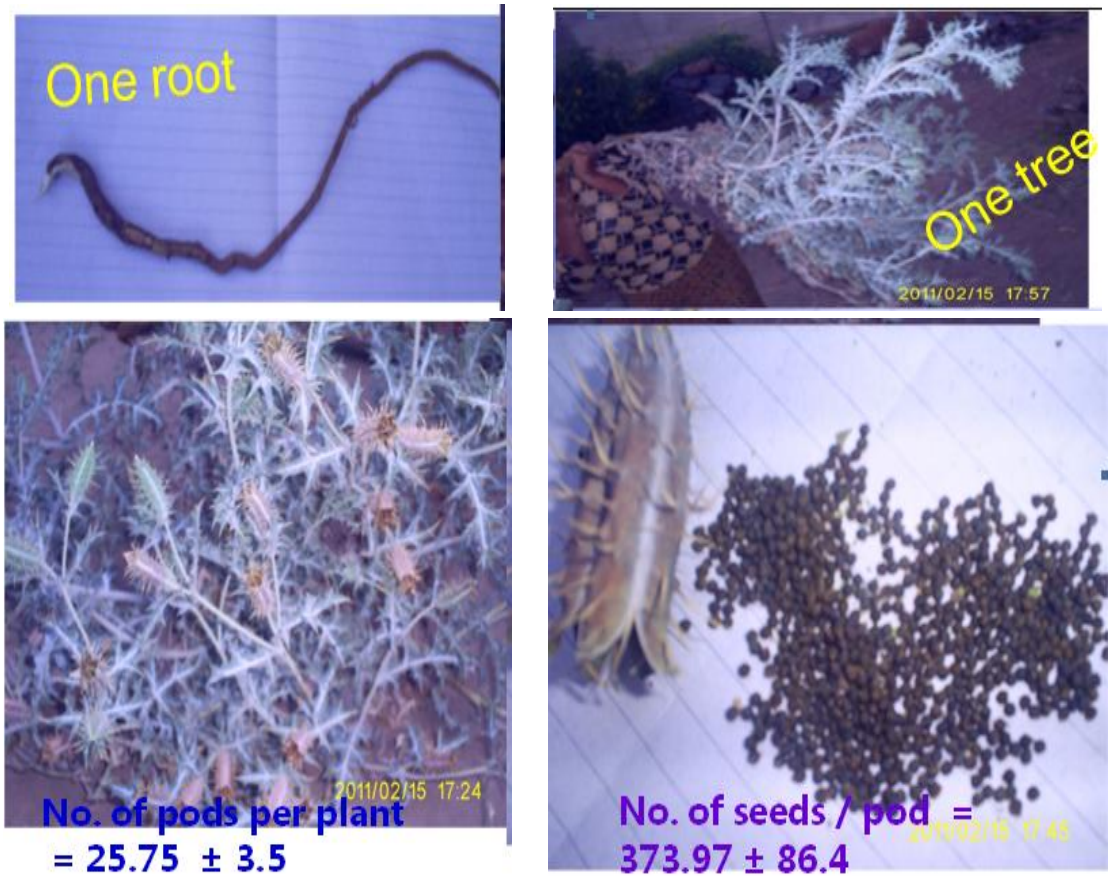

Fig. 4. The number of seeds per pod and pods per plant

The invasive alien species have invaded many ecosystems and communities throughout the world, disrupting ecosystem structure, function and reducing native biodiversity. *Argemone* is doing the same in Ethiopia. *A. mexicana* has a strong dormancy level that only a fraction of the seeds produced would germinate at any one particular time, irrespective of pretreatment but a substantial part of the *Argemon*e seeds that ripe every year will enter into the seed bank, and will probably germinate in an unpredictable pattern during several consecutive years [12]. Therefore, 7000 -9000 seeds that one plant has, can have a chance of germinating and cover many land use types.

A number of plant secondary metabolites like alkaloids and flavonoids have been reported as anticancer, antiviral, antibacterial and antiamoebal agent [15,6,5,14]. The broad antimicrobial activities of this extract could be as a result of the plant secondary metabolites [13] and hence the presences of tannins, steroids, and flavonoids in the seeds of *Argemone mexicana*. While glycosides and saponins have antiprotozoal activity of *A. mexicana* revealed the presence of labdane type diterpene compounds. These compounds are similar to those that have been reported to possess strong antimicrobial activity [14]. The Tannins, flavonoids, steroids are known to have curative activity against several pathogens. Both the seed oil and leaf infusions of *A. mexicana* are drunk to relieve cough; root and leaf decoctions are applied to the skin to care edema, inflammation, muscle pain, ulcers and yaws [15]. Therefore, *Argemone* could be suggested for the treatment of various diseases.

5. CONCLUSION

A. mexicana invaded many ecosystems and communities in Ethiopia in general, Farta, Gondar and Bahir Dar zuria districts in particular disrupting ecosystem structure, function and reducing native biodiversity (plants and animals). When the distribution and abundance of *A. mexicana* increased the abundance and distribution of native plant species decreased. *A. mexicana* shown excellent potential of antibiotic activity towards the bacteria *S. aureus, S. agalectiae and E. coli* and inhibits its growth. *Argemone* has aflatoxins that often occur in crops in the field prior to harvest. Post harvest contamination can occur if crop drying is delayed and during storage of the crop if water is allowed to exceed critical values for the mould growth. Milk, eggs, and meat products are sometimes contaminated because of the animal consumption of aflatoxin-contaminated feed. Therefore, the health of the animals are at risk as the distribution increases besides it biodiversity destruction.

6. RECOMMENDATION

1. Bureau of Agriculture in particular the pertinent GOs and NGOs should find a mechanism to eliminate this invasive plant and save the farm and grazing lands before becoming uncontrolled.
2. Further investigation is need to investigate the use of *Argemone* oil for industrial purposes
3. Further investigation should be undertaken at molecular level so as to identify gene – anti-gene reaction between the bacteria and *Argemon*e extracts for further pharmacological application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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