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Awareness of people living in Sagure District, Arsi, Ethiopia about ergot fungus (*Claviceps purpurea*) and ergotism

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ABSTRACT: Ergot is a plant parasitic fungus that belongs to the genus *Claviceps*, and ergotism is a poisoning caused by secondary metabolites produced mainly by the ergot fungus (*Claviceps purpurea*). The presence of ergot fungi in the farmers' field was surveyed, and ergot sclerotia were clearly observed in the farmers' fields on wild oat plants. A cross-sectional study was therefore conducted in three Kebeles of the Sagure District (Kechema Murkicha, Digelu Kidame and Mankula Negele), near Asela town, Ethiopia, to assess awareness of the residents about ergot fungus and ergotism. From a total of 385 study participants only 100 (26%), knew what ergot and ergotism stand for. Thirty-one percent of the study participants described the most common symptoms of the ergot poisoning as 'cutting off legs' and removing wild oats (Avena abyssinica), the host for the ergot fungus, as the main method of prevention of the disease. Among the socio-demographic factors, education level was found to be significantly associated with the knowledge of ergot (p < 0.001), where participants from secondary education and above had better knowledge about ergot fungus and ergotism (COR: 2.5, 95% CI:1.34 - 6.22). From binary logistic regression model fitted, study site has statistically significant association with knowledge about ergot (AOR: 5.34, 95% CI:3.25 - 8.43, p< 0.001). In conclusion, though the fungus was prevalent in the farmers' fields, majority of the study participants were not aware of ergot fungus and ergotism, thus there is a need to create awareness about the fungus and the disease in order to prevent possible future disease outbreaks.

Key words/ phrases: Arsi, Awareness, Claviceps purpurea, Ergotism, Wild oats.

INTRODUCTION

Ergot is a parasitic fungus that belongs to the genus Claviceps (Nicholson, 2007). The genus *Claviceps* parasitizes more than 600 monocot plants including economically important crops such as rye, barely, oat, rice, wheat, pearlmillet (Bove,1970) and wild oat (Avena abyssinica) (Teshome Demeke et al., 1979). The name 'ergot' is derived from an old French word 'Argot' which means Cock's spur which represents the dark brown, horn-shaped fungal structure that projects from the ripening ears of infected crops replacing the grains (VanDogen and DeGroot,1995; Nicholson, 2007). Ergot is the overwintering sclerotia of the fungus (Claviceps) formed at the end of the infection process by sexual spores asexual (ascospores) (conidia) or spores (Alderman, 2003).

The sclerotia of the ergot fungus are composed of secondary metabolites known as ergot alkaloids.The main ergot alkaloids produced by *Claviceps* species are ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine In France and other European countries west of the Rhine River, outbreaks of ergotism were generally of the gangrenous type, whereas in Central, Eastern Europe and Scandinavia, outbreaks were of the convulsive type (DeCosta,

and their isomers (EFSA, 2005). Ergot alkaloids are toxic metabolites that were responsible for the mass poisonings in the middle ages in Europe due to consumption of bread made from rye contaminated with sclerotia of the ergot fungus (Barger,1931). The disease that results from consumption of foods contaminated with the ergot sclerotia containing the toxic ergot alkaloids is ergotism. There are two forms of the disease, the gangrenous ergotism also known as "St. Anthony's fire" or "Holy Fire" and convulsive ergotism "St.Vitus' Dance".The gangrenous ergotism is characterized by an intense burning pain resulting from vasoconstrictive effects of the ergot alkaloids and subsequent loss of fingers, hands, feet and even entire limbs (Gabbai,1951; Kelbessa Urga et al., 2002). The convulsive forms were characterized by symptoms such as hallucination, delirium and Epileptic-type seizures (DeCosta, 2002; Kokkonen and Jestoi, 2010).

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2002; Edie, 2003). Gabbai *et al.* (1951) observed that the epidemic of ergot poisoning occurred in France, symptoms began to appear on August 1951, following aperiod of 6-48 hrs after consumption of the ergot contaminated rye bread. During this epidemic 25 cases were of severe delirious forms and four cases comprising of 3 men and 1 woman died of cardiovascular collapse.

In Ethiopia, gangrenous ergotism was reported by Teshome Demeke et al. (1979) in Waro and Gazo-Belay areas of the highlands of Lasta and Wadla Delanta, Northern part of Wollo. According to this report, the epidemic occurred due to consumption of locally grown barley contaminated by ergot sclerotia on wild oats. Atotal of 93 cases and 47 deaths of ergotism were reported, and majority of the cases were between the age of 5 and 34 years. The symptoms of the disease ranged from the general symptoms such as weaknesses, nausea, vomiting and diarrhea to dry gangrene of whole or part of the limbs. The most recent out break of gangrenous ergotism in human history occurred in 2001 in Tijo and Digelu areas of the Arsi zone, Ethiopia, following consumption of barley containing ergotized wild oat. During this out break 18 cases aged between 5 to 30 years were affected by the disease with three deaths (Kelbessa Urga et al., 2002).

The aim of the study was therefore to assess the level of awareness of the local community living in the most recent ergot poisoning outbreak areas of the Arsi, zone, Ethiopia.

METHODOLOGY

Description of the study area

The survey was conducted in three Kebeles (the smallest administrative units) of the Sagure District of the Arsi zone, namely Digelu (Digelu Kidame) Tijo (Mankula Negele) and Kechema areas (Kechema Murkicha) of the Arsi zone, Ethiopia during the month of May 2017. Tijo (Mankula Negele) is located about 50Km from Assela, Digelu (Digelu Kidame) is located about 50Km from Assela and Kechema (Kechema Murkicha) is located about 38 km from Assela town

Research Design

The study employed both quantitative and qualitative research designs. Closed and open ended questionnaires were used as instrument for gathering information from the study participants

Quantitative Research Design

Across-sectional research design (Nee and Sani, 2011) was conducted in three different Kebeles of the Sagure district namely Mankula Negele (Tijo area), Digelu Kidame (Digelu area) and Kechema Murkicha (Kechema Murkicha area), Arsi zone, Ethiopia.

Qualitative Research Design

The qualitative information was gathered through focus group discussion, key informants interview and snowball sampling protocols (Robert, 2011).

Sample size determination

A total of 385 study participants took part in the current study, which was designed to assess the level of awareness of the local community about the "ergot fungus" and the disease "ergotism". The sample size for this study was calculated using the formula indicated below with 95% CI, 5% marginal error and 50% estimated level of awareness of the study participants about ergot fungus and ergotism (Cochran,1963).

 $N=Z^2p(1-p)/e^2$

Where, N=required sample size

Z=confidence level at 95% (1.96 standard value) p=estimated awareness in the study area(0.5) e=marginal error at 5%(0.05 standard value) or the level of precision.

Sampling

Quantitative study

The sampling sites were divided into three areas namely Digelu, Tijo and Kechema areas based on proximity of the Kebeles to each other. The Digelu and Tijo areas are at relatively higher altitude compared to Kechema area. Three Kebeles namely, Digelu Kidame, Mankula Negele and Kechema Murkicha were randomly selected by lottery method from Digelu, Tijo and Kechema areas, respectively. After the Kebeles were selected the total population size of the selected Kebeles was obtained from the administrative heads of each Kebele and proportion of the study participants was estimated. Accordingly,110 study participants from Digelu Kidame (Digelu area), 110 study participants from Mankula Negele (Tijo area) and 165 study participants from Kechema Murkicha (Kechema area) were randomly selected. Questionnaires were delivered to the selected house holds by data collectors trained for one day and the principal investigator.

Qualitative study

For qualitative study, purposive sampling was used to recruit participants to the study. To gather information regarding the ergot fungus and ergotism from the community, key informants interview and snowball sampling were used. The focus group discussion was conducted with health professionals from Assela Hospital comprising of (4 medical doctors and two public health officers) from the department of internal medicine. Key informants interview was conducted with community members who had prior training on the ergot fungus or had first hand information about ergot fungus and ergotism, as well as a staff of the department of agriculture located at the Sagure town. Snowball sampling was used to gather information from the cases of ergotism from Tijo areas to assess their level of awareness about the disease.

Ethical clearance

Ethical clearance was obtained from the College of Natual and Computational Sciences, Addis Ababa University Institutional Review Board. Before data collection, consent for participation was obtained from all the study participants. The purpuose of the study, the right to participate and decline at any time during the study were informed to the study participants. The study participants were also informed about the confidentiality of the data they provide during the survey and that the data was only be used for the research prurpose.

Data analysis

All of the completed questionnaires were coded and entered into SPSS version 24. Frequency and percentage were used for descriptive analysis and logistic regression was used to examine the association between some important variables and knowledge about the ergot fungus.

RESULTS

Quantitative study

The majority of the study participants in the current study were males (87.5%), married (98.7%), farmers (96.9%) and lived at the study area for more than 16 years (98.7%). About 46.2% of the study participants were between the age of 30 and 41. Nearly 51 % and 42.6% of the study participants were chritians (51) and had 3 - 5 family size (Table 1.).

Table 1. Socio-demographic characteristics of the study particpants.

Variables	Frequency	Percent
Sex		
Male	337	87.5
Female	48	12.5
Marital status		
Married	380	98.7
Single	4	1
Widowed	1	0.3
Age		
18-23	5	1.0
24-29	46	12.0
30-35	89	23
36-41	89	23
42-47	53	14
48-53	46	12
54-59	25	7
60 and more	32	8
Education level		
Illitrate	40	10.4
No formal education but can read and write	50	13
Primary education	220	57.1
Secondary eduction and above	75	19.5
Occupation		
Farmer	373	96.9
Merchant	4	1.0
Government employee	1	0.3
Self employee	5	1.3
Student	2	0.5
Religion		
Chritian	196	51
Muslim	187	49

From the demographic-factors used in the current study, statistically significant association (p < 0.05) was found between education level and awareness of ergot where respondents from higher education category had more knowledge about ergot and those with lower education levels had less knowledge about ergot. However, based on the multiple logistic regression model fitted, taking variables associated with the response from univariate analysis, only study sites had statistically significant association (AOR:5.34, 95% CI:3.25 - 8.43, p< 0.001) with the study participants' knowledge of ergot. Better awarness of the study participants about the ergot fungus was recorded from Kechema Murkiacha followed by Mankula Negle and the least knowledge about ergot was recorded from Digelu Kidame.

From the 385 study participants recruited from the three Kebeles (Digelu Kidame, Kechema Murkicha and Mankula Negele) only 26% reported that they had heard of ergot and ergotism, and 30.6% of the study participants knew a person or family affected by ergotism (Table 2). Regarding the common symptoms of the disease, the study participants responded that the disease " cuts off legs" 31 (8.1%), "cuts off arms" 1 (0.3%), " blackening, wounding, drying, severe pain of the legs and lack of sleep" 5(1.3%), "swelling of limbs" 25 (6.5%), "headache" 2 (0.5%) and "nausea, vomiting, diarrhea and stomach ache" 26 (6.8%).

After showing the coloured picture of the ergot fungus, relatively higher percentage (43.6%) of the study participants responded that they recognized the picture (Table 2). Then they were asked about the local name of the fungus in the picture " Ergot picure", 55 (32.7%), answered "Sinara guracha" or " Black wild oat", 25 (14.9%), "Sinara Dama" or " Honey wild oat", 23 (13.7%), " Dhukuba Sinara" or " The disease of wild oats", 22 (13.1%), "Dhufiyee" which based on the discription fits to "Smut", 39 (23.2%) of the study participants either don't know or 4 (2.4%) didn't give response.

Majority 100 (59.5%) of the study participants saw the fungus in their farm and 5 (1.3%) did not give any response. If they saw it in a farm, they were asked on which crop they saw the fungus indicated in the coloured picture, majority 129 (33.5%) answered that they saw it on wild oats, 4(1%) on wheat, 1 (0.26%) on barely and 1 (0.26%) did not give response(Table 2).

Table 2. Awarness of the study	v participants about	ergot fungus and	d ergotism, and the	ir preventive practices.
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No	Questions	Responses	Frequency	Percent
1	Have you heard of the term ergot?	Yes	100	26
		No	285	74
2	Have you heard of the disease ergotism?	Yes	99	25.7
		No	286	74.3
3	Do you know a person or a family affected by ergotism?	Yes	30	30.3
		No	69	69.7
4	Do you think the disease is transmissible?	Yes	2	2
		No	72	72.7
		I don' t know	25	25.3
5	Do you think the disease is preventable?	Yes	65	65.7
		No	30	30.3
		I don't know	4	4
6	Do you know what this picture represents? (Respondents' were shown the colored picture of ergot fungus)	Yes	168	43.6
		No	217	56.4
7	Where did you see it?	In farm (a)	100	59.5
		In harvested grains (b)	28	16.7
		In both (ab)	35	20.8
		No response	5	3
8	If in farm on which crop?	Wild oats	129	95.6
		Wheat	4	3
		Barley	1	0.7
		No response	1	0.7

Among the study participants who recognized the picture of ergot, 142 (84.5%) said that farmers remove the ergot from crop fields or harvested grains (Table 3) by hand picking 99 (25.7%), cutting the ergot and fungus together with wild oats using sickles 15 (3.9%). About 28 (7.3%) didn't answer 19 (2.3%) or provided other responses 9 (2.3%).

Majority of study participants 224 (65.9%) responded that farmers remove wild oats, by hand picking 120 (47.2%), hand weeding and using herbicides 89 (35%), using herbicides 24 (9.5%),

cutting wild oats 7(2.8%) or using the combination of all the three methods 8 (3.2%) and 6 (2.4%) of the study participants didn't describe how farmers remove the wild oats from their crop fields (Table 3). They also indicated that wild oats are removed from agricultural fields when they flower and are distinguishable from other crops or using herbicides after the wild oat emerges 170 (44.2%), or when the crop matures 19 (4.9%), 1(0.3%) unrelated response and 64 (25.2%) didn't give any responses.

Questions	Responses	Frequency	Percent
Do farmers remove ergot from their crop fields?			
	Yes	142	84.5
	No	25	14.8
	No response	1	0.6
How do they remove it?			
	Hand picking	99	69.7
	Cutting with sickles	15	10.6
	Others	9	6.3
	No response	19	13.4
Do farmers remove wild oats from their crop fields?			
	Yes	254	65.9
	No	128	33.2
	I don't know	1	0.3
	No response	2	0.5
How do they remove the wild oats?			
	Hand weeding (a)	120	47
	Using herbicides (b)	24	10
	Using both (ab)	89	35
	Cutting with sickles (c)	7	3
	Combination of all (abc)	8	3
	No response	6	2

Table 3. Farmers practice regarding removal of ergot from their crop fields.

a, hand weeding; b, using herbicides; c, cutting with sickeles; abc, using all the three methods

Key informants interview

Knowledge of the ergot fungus and the disease ergotism varied over the key informants interviewed. However, the response of majority of them regarding the description of the disease symptoms was similar and was as described below.

"I know ergot, it is the name of the blackwild oats that produces honey on the head of wild oat plants. In the study area it is generally called honey wild oat or wild oat with honey". If this type of wild oat is consumed with food it can cause a disease that leads to loss of legs by sucking blood out of the big toe and gradually separating the flesh from the bone. The disease symptoms start with the big toe; first it sucks blood out of the big toe and dries it up leading to very painful burning sensation and sleeplessness."

"The disease occurred in our neighborhood, Shaldo Jigessa Kebele. Initially we thought it was caused by bad spirit that targeted some family. Later on people from the health center gave us training and we knew that it was caused by the black wild oats with sticky substances or glue"

Regarding the source of information, transmission and prevention of the disease, majority of the key informnts said:

"The disease occurred in our localities of the Tijo areas 'Tite Waji', 'Mankula Negele' and 'Shaldo Jigessa'. It cannot be transmitted from patients to healthy person and it is possible to prevent the disease by removing black wild oats or honey wildoats from harvested grains or crop fields".

Three of the key informants, however, differed in response regarding prevention of the disease. The responses were " It is possible to prevent the disease by taking great care not to touch the ergot fungus", "The disease cannot be prevented" and "It is possible to prevent the disease by eating balanced diet". Majority of the key informats also said " It is necessary to remove the eregot from the grains and agricultural harevested fileds. Regarding prevention practices, most of the key informants reported that the common practice in the area was weeding out the wild oats giving it to their cattle as feed. They also reported that the disease "ergotism" is meerly the disease of humans and doesn't affect their cattle. Few of the key informants also reported that if the cereal is for market purpose the black wild oat "ergot" is not removed from the cereals.

Regarding the prevalence of the "black wild oat" or the "ergot". Most of the key informants said they don't know why it occurs in the farmer's fileds , though it is found in the fileds every year. However, the highest prevalence of the "ergot" according to the key informants occur when the rainy season was prolonged. The reason " ergot" is commonly found in the crop fileds, according to some key informants is due to ineffectivness of herbicides intended to remove the wild oats from barley and wheat fileds. This is because the farmers over dilute the herbicides to cover more of their crop fileds and due to shortage or delayed supply of herbicides. The other reason for ineffectivness of herbicides is due to improper timing of herbicide application where early or late application led to its less effectivess"

Focus group discussion

The focus groups were asked whether they know ergot fungus and ergotism. All of them answered "We have never heard of ergot and ergotism". They were then asked if they know what ergot alkaloids are and most of them reported "ergot alkaloids are pharmaceutically important chemicals such as ergometrine which are used to prevent post partum hemorrhage." The group was asked about the cause of gangrene, if they know any case of gangrene in the area and how they were treated.

"Gangrene can be caused by toxins from different bacteria. When we were in the OPD there

were two cases of gangrene in the area and both were women. One of the two women had acute onset of gangrene and the other women was chronic gangrene case. The woman with chronic gangrene was referred to the Black Lion Hospital." Ergot alkaloids were not considered as the possible cause of the gangrene in the two cases during the morning report." Most of the focus group also reported the use of hyperbaric oxygen and amputation as the possible treatment of the gangrene.

Snowball analysis

This report is based only on information gathered from one case of ergotism, as the cases were not willing to praticipate in the study, to assess his overall awareness about the disease. He responded to all the questions saying "Ergot the black wild oat. It is a very dangerous disease that results due to consumption of food contaminated by black wild oats. It occurred in Mankula Negele, Tite Waji and Shaldo Jigessa Kebeles. The disease symptoms initially started from my big toe with burning sensation; it sucked blood out of my toe and dried it up. It was very painful and was even hard to explain. I didn't have sleep the whole night and day. The disease is not transmissible and it is possible to prevent it. The disease can be prevented by using balanced diet and removing wild oats from agricultural fields."

DISCUSSION

In the current cross-sectional study about ergotism in the highlands of Arsi, Ethiopia, the awareness of a total of 385 study participants from three Kebeles under Sagure district was assessed. The finding of this study showed that there was limited awarness about the ergot fungus and ergotism. Majority of the study participants (97%) were farmers. Similarly Ephrem Guchi et al . (2014), also found out that 98.7% of farmers, 96.7% traders and 70% consumers were unaware of aflatoxin contamination and its consequence. All (100%) of the farmers participated in the study by Ephrem Guchi et al. (2015), never heard about aflatoxin. This comparison is made due to lack of information about survey of ergotism in Ethiopia, eventhough it is not appropriate due to differences in the study topic and population.

The differences in the level of awareness between our study and study by Ephrem Guchi *et*

al.(2014) could be due to differences in the study populations and the topic of the study. The lack of awareness of majority (74%) of our study participants about the fungus and the disease might be due to loss of memory since the disease outbreak occurred in the area only once and was about 15 years ago. There is high likelyhood of loss or reduction of memory of past events due to aging (Small, 2002).

Among the study participants who were aware of the ergot fungus and ergotism(26%) majority of them described the local name of the fungus as "Sinara Guracha" which means Black wild oat. This might be due to the black coloured sclerotia of the ergot fungus (Van Dongen and De Groot, 1995)

Some also described it as "Sinara Dama" which means honey wild oat; this is also due to the fact that fungal infection of the flower head of the wild oat plants leads to oozing of sugary substances that contains sucrose, glucose, fructose and the asexual spores of the fungus known as "Honeydew" (Alderman, 2003). Some of the study participants also described the ergot fungus as "Dhukuba Sinara" which means the disease of wild oat. This is also supported by Bove (1970), who described ergot fungus as the disease of more than 600 monocotyledon plants including rye, barely, oat, rice, wheat and millet. There were also misconceptions about the ergot fungus where some of the study participants describe the ergot fungus as"Dhufiyee" which based on the description by the study participants fits to "smut". This misconception might be due to the blackish appearance of both fungi on the heads of crops.

Among the study participants who were shown the coloured picture of the ergot fungus, majority of them said they saw it on wild oats which is also in agreement with Teshome Demeke et al.(1979) who reported about ergotism in highlands of Wollo, Ethiopia due to consumption of barely contaminated by ergot fungus from wild oats. Kelbessa Urga et al. (2002), also reported ergotism in the highlands of Arsi that might be due to consumption of food contaminated by ergot sclerotia from wild oats. Besides, the presence of ergot sclerotia on wild oat plats was confirmed from the five years (2011 - 2015) survey visits to crop fields in the study area by the principal investigator of the current study. The symptoms of ergotism were described by the study participants as "cuts off legs", "cuts off arms", "Blackening, wounding, drying, severe pain of the legs and lack of sleep", "Swelling of limbs", "Headache" and "Nausea, vomiting, diarrhea and stomach ache". Some of these disease symptoms were also reported by (KelbessaUrga *et al.*, 2001).

According to Rey et al. (2003) observation, the most common signs of ergot poisoning include gastro intestinal symptoms such as nausea, vomiting and abdominal pain and neurological symptoms such as headache, dizziness and decreased level of consciousness. Symptoms such as intense pain resulting from vasoconstriction and subsequent gangrene with loss of fingers, hands, feet and even entire limbs, vomiting and insomnia (Gabbai, 1951) are also in agreement with our hallucination which studv. However, was described by the same author does not fit to the description of the disease symptoms in the current study. This might be because, the symptoms of ergotism that occurred in Arsi, highlands was typical symptoms of gangrenous ergotism, and hallucination is one of the symptoms of convulsive ergotism (De Costa, 2002).

Regarding the prevalence of ergot fungus in the study area, majority of the key informants did not know why the prevalence of the fungus is different from year to year. But, one key informant said "Prevalence of ergot in the study area is higher during prolonged period of rainy season." Similarly, Craig and Hignight (1991), stated that the prevalence of ergot species is dependent on climatic conditions and especially pronounced during seasons with very heavy rainfall and wet soil conditions. This might be because wet soil conditions facilitate the formation of sexual structures that produces infective sexual spores that can forcefully disseminate to infect more flower heads of the host plants.

Of the 65 study participants who think the disease is preventable, majority of them 35 (9.1%) said that the disease can be prevented by removing wild oats from agricultural fields and 15 (3.9%) by removing the black wild oat "ergot" from harvested grains before going to Mill house. This practice is also in agreement with Posner and Hibbs (1997), who reported that effective cleaning techniques at the mill houses enabled removal of 82% of the sclerotia from grains. The cleaning procedure however becomes less reliable when the intact sclerotia are broken into fragments or small sclerotia with similar size to the grains are produced due to dry environmental conditions (Lauber et al., 2005). Even when the ergot sclerotia are completely removed, ergot alkaloids can still

be detected in food and feedcommodities (EFSA, 2005).

Among the socio - demographic factors in the current study, education level is significantly associated with Knowledge of ergot ($X^2 = 13.34$, p = 0.004). The knowledge about ergot was the highest for the study participants with secondary education and above. This is also in agreement with a study by Ephrem Guchi et al. (2014), where respondents from higher education level had better awareness than those from lower education level. People with higher education level were also found to be positively related to some types of risk of food or pesticides in food than those from lower education levels (Dosman et al., 2002). This might be because people with higher education levels might be better informed than those from the lower education categories.

Based on the multiple logistic regression model fitted taking variables associated with the response from univariate analysis study site was found to be significantly associated with the knowledge of ergot (P<0.001). This might be because of awareness trainings given in the area by the staff of the department of agriculture who is still working in the area of Kechema Murkicha. These highest level of knowledge about ergot fungus was observed among the study participants recruited from the Tijo area (Mankula Negele) this might be due to the occurrence of the disease in the area. The information for the current study was based on an outbreak which occurred about 15 years back, for which there mightbe loss of memory of the disease condition as it occurred only once in the area. Besides, only one case of ergotims was willing to participate in the current study though four of them were communicated.

CONCLUSION

In this study majority of the study participants didn't know about ergot and ergotism, despite the occurrence of the disease outbreak in the study area. Even among the study participants who said they know about ergot fungus and the disease ergotism, there are some misconceptions regarding their knowledge of ergot where some study participants associated the ergot fungus with smut. Generally, misconceptions were observed among the study participants about ergot fungus and the disease ergotism. Thus, creating awarness about the fungus and the disease by the concerned body is cruicial to prevent the possible future outbreak.

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