Online ISSN : 2249-4626
Print ISSN : 0975-5896
DOI : 10.17406/GJSFR

Global Journal

of Science Frontier Research: D

Agriculture & Veterinary

Agronomic Evaluation of Coffea

Bacterial Causes of Bovine Mastitis

Highlights

Effect of Mycorrhizal Inoculation

Integrated Management against Seed

Discovering Thoughts, Inventing Future

VOLUME 18 ISSUE 4 VERSION 1.0

Global Journal of Science Frontier Research: D
Agriculture & Veterinary

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Agriculture & Veterinary
Volume 18 Issue 4 (Ver. 1.0)

Open Association of Research Society

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

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Framingham Massachusetts Pin: 01701,
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USA Toll Free: +001-888-839-7392
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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 4 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Agronomic Evaluation of *Coffea Arábica* Variety Castillo and Caturra in Two Production Systems (Sun and Shade); In the Los Naranjos Farm, La Venta (Cajibio-Cauca)

By Montoya Bonilla Bibiana Patricia, Ordoñez Fernández Zulma Katerine & Bonilla Blanca Lilia

University Corporation Comfacauca-Unicomfacauca

Abstract- In Cauca, coffee is prioritized in the competitiveness agenda, as a core of innovation to strengthen the appellation of origin; This study evaluates the behavior of the Castillo and Caturra varieties in two production systems, sun and shade determined by lustiness, production and flowering variables, as well as establishing the effect of the agronomic conditions on the crop.

The experimental design selected Castillo and Caturra varieties, allowing the evaluation of 40% of plants and monitoring of the variables, using own scales with categories that increase from 1 to 4; the recording and analysis of information used the Excel platform, applying statistical methods of distribution and measures of central tendency, as well as significant evaluation.

This is how the statistical process shows favorable results for the Caturra variety with higher percentage of lustiness (90% L1) and flowering (40% L2), but a smaller difference in production (30% L1) compared to Castillo (37% L4).

Keywords: coffee, production system, castle, caturra.

GJSFR-D Classification: FOR Code: 070302

Strictly as per the compliance and regulations of:

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Montoya Bonilla Bibiana Patricia [α], Ordoñez Fernández Zulma Katerine [σ] & Bonilla Blanca Lilia [ρ]

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I. Introduction

Coffee is considered one of the main economic and social products offered by the country thanks to its quality [1], but this is also determined by the different conditions of the agro-ecosystems that are formed coffee, it is clear that the cultivation of coffee is grown in about 80 tropical and subtropical countries around the world and its main distribution is found in small and medium-sized farms of around 10 Ha [2] directed by coffee growers that promote family subsistence; However, despite the fact that coffee is considered a core productive bet within the Internal Agenda of Productivity and Competitiveness of the Department of Cauca, it has been identified that one of the greatest challenges to which this nucleus is exposed is that producers have access to the different certification programs required by international markets [3], thus, many of the challenges for strengthening the production and marketing of certified regional coffees have been somewhat delayed [4].

At the local level, the Cauca coffee is among one of the main departments in the production of high quality special coffees with denomination of origin, accompanied by Santander, Huila and Nariño; its production is 100% smooth Arabica and its processing is linked to methodologies and protocols determined by the National Federation of Coffee Growers, transferred to the producers by its group of rural extension agents, as well as the Cauca coffee with its Cauca Denomination of Origin, and where it is recognized by characteristics such as: "a coffee with fragrance and aroma very strong and caramelized, which in cup presents high acidity, medium body, balanced global impression, clean, soft with some sweet and floral notes" [5] seeks to contribute day a day to achieve standardization in production and processing, since its levels of consistency and homogeneity is what has made this coffee to be evaluated by the markets and the most demanding palates.

It should be noted that all the characteristics and attributes acquired by coffee as a final product are linked to the interaction of several components within a system; where its complexity depends on the number of related factors; their study is general but their interrelations tend to serve the same purpose; as for example in the present study we have two types of production systems; the first a production system with semi-shade and the second a production system with free solar exposure; both include within their system other subsystems such as weeds, climate, soil and coffee plants and others; this means that the study of all these interactions contributes to the final characteristics of the product [6].

It is here where the National Federation of Coffee Growers and Coffee Research Centers have determined that coffee growers have become the main players in this chain and where research and work in coffee production systems under the different production units and The environment formed between each of them are key to achieve recognition,

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strengthening, competitiveness and high levels of quality. Therefore, the present research study evaluated the incidence of agro forestry systems with coffee and production systems for free solar exposure in two varieties of Castillo and Caturra coffee, thus identifying the agronomic aspects that influence the production of coffee as the lustiness, production and flowering periods; study that contributes to the coffee chain with baseline information for agronomic studies of variety behavior and its response to a variety of agro-ecological and micro-climatic conditions offered by the Popayán Plateau.

II. Materials and Methods

Study area: The research area was carried out at Hacienda los Naranjos, owned by SUPRACAFE, located in the village of La Venta, Cajibio Cauca municipality, geographically positioned at coordinates N2 35,086 W76 32,959 (Figure 1), and located approximately at 1862 masl, on the banks of the Purace volcano on volcanic soils, with a distance of 28 km from the city of Popayán, with characteristics of flat topography, arboreal vegetation to its surroundings, and where its focus has been determined in the production of coffee, with its own farms converted into technological validation stations, where in conjunction with various entities and universities, more than 200 varieties of Arabica coffee are being validated and micro-lots of high quality and traceability for international markets are being produced.

Source: Authors edited by Google Maps, 2017

Fig. 1: Research Area Location

Methodological Design: For the research, a selection of 4 lots was made, two for each variety and production system, characterizing 50 continuous plants for each batch, with a distance of 1.5 m between rows and 1.3 m between plants; in the lots 40% of the 50 plants corresponding to 20 plants per lot were evaluated with a signaling criterion called Five of golds [7], which covers a marking of 4 plants for each of its corners and 4 plants allowing so perform a systematic evaluation of the lot. In addition, for each of the plants for monitoring, two branches were taken in different parts of the plant [8], whether primary or branched, superior, inferior or intermediate; this in order to identify the aspects to be evaluated throughout the plant.

Registration of Information: The information was consolidated in data recording formats taking into account some time intervals depending on the format to be evaluated, and the value of the variable was determined on scales of value from 1 to 4, with 1 being the lowest and 4 the largest, and indicating a scale number for each of the variables evaluated as shown below:

Flowering: Evaluating in each of the selected plants the branches that have been marked initially and determining in what stage of flowering it is (E1, E2, E3, E4,) adapted by [8]. Production: In which the amount of grains produced in each of the branches marked by each plant under evaluation is monitored. Phytosanitary control: Format that allows to determine the status of plants with respect to lustiness, the incidence of pests or diseases and well-marked deficiencies of nutrients. Climatic conditions: A record of climatic conditions is made, for example: Relative Humidity, Ambient Temperature; which allow to determine some variations within the research area and with it to be able to determine its influence with the agronomic behavior of the plants under study.

Analysis of the Information: The information registered for the variables was tabulated and developed by means of analysis of significant differences from the evaluation of the variables and their behavioral tendencies, in addition to the correlation of the parameters of development of the plant with respect to agroecological tensors.

III. Results and Discussion

Characteristics of the Lots: Information was obtained on the characterization of each of the lots under investigation; corresponds to the identity of each lot and which are the variables that act directly on them as shown in the following table:

Table 1: Characteristics of the Lots in Research

# Lots in Research	1	2	3	4 (3N)
Variety	Caturra	Caturra	Castillo	Castillo
Production System	Partial Shade	Sun	Sun	Partial Shade
D.S Grove	1,5 m	1,5 m	1,5 m	1,5 m
D.S Plant	1,3 m	1,3 m	1,3 m	1,3 m
Sowing Date	1-Jan-2009	1-Feb-2009	1-Mar-2009	1-Apr-2009
Last Fertilization	Oct – 2016	Oct – 2016	Oct – 2016	Oct – 2016
Renovación	Bandola	Bandola	Calavera	Without Renewal
Last Flowering	12-09-2016	12-09-2016	12-09-2016	12-09-2016

Source: Authors

The results shown below are a collection of data enclosed in a tabulation of scales where the behavior of the variables is expressed with the most representative scale, that is, the one that allows to identify the optimal values in the variables under study, in which An analysis is made of each of the variables such as lustiness, flowering and production in the plots.

Table 2: Behavior of Lots in Research Variables (%)

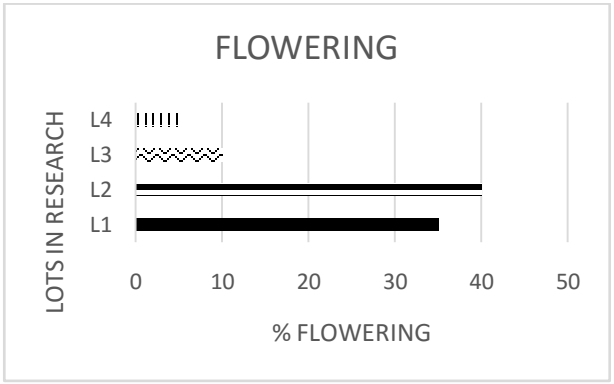
Lots	Lustiness	Flowering
L1	90	35
L2	88,3	40
L3	61,6	10
L4	88,3	5

Source: Authors

Lustiness: Aspect of great importance that allows to identify in a visual way the state of the plant; evidencing the response of the same to different environmental factors and to the production systems to which they are exposed.

Graph 1: Major Scale Lustiness Variable

Flowering: In the cultivation of coffee a series of periodic biological changes occur, but there are some more important than others, for example, flowering, reproductive formation of coffee that acts as indicators of reproduction and productivity phases [9].



Graph 2: Variable of Flowering in Greater Scale

Production: Stage of great interest for coffee farmers, the period of post harvest that is made to this production is one of the most important determinants for coffee quality; then, in table 3, the production ratio per plant and its weight is shown.

Table 3: Production Ratio

Lote	% Pn	# Grains	P. Prom. Grains	Pn*Grs L.sample
1	15,9 %	1399	1,93 gr	2,7 kg
2	30,2 %	2657	2,27gr	5,8 kg
3	16,5 %	1452	1,77gr	2,5 kg
4	37,2 %	3271	2,41gr	7,8 kg

Source: Authors

IV. Discussion

Lustiness: As can be seen in graph 1, the behavior of the lustiness variable (Scale 4) in the plots under evaluation corresponding to larger leaves of dark green color, brightness in its beam, firm, healthy, without affectations and healthy fruits showed its greater percentages of better lustiness in lot 1 (shade - Caturra) with 90%, followed by lots 2 (sol - Caturra) and 4 (shade - Castillo) with similar percentages of 88.3% and finally the lower lot Strength percentage in scale 4 was lot 3 (sun - Castillo) with a percentage of 61.6%, identification determined from an analysis of percentage averages.

In conclusion in the batch of the system of partial shade production that presented better percentages of lustiness where the characteristics of the shadow act hand in hand with the direct influence of the micro climatic and agroecological conditions that has favored the behavior of the variable lustiness previously mentioned, where it is attributed to what contributes to the disposal of organic matter, the assimilation of nutrients, soil protection, the action as a filter against solar radiation, root protection, harvest quality, among others; [10] and [11]; and the lower lustiness in the batches of the production system with direct exposure to the sun, which, despite being varieties developed to adapt to solar exposure conditions, are affected to a minimum by these conditions. On the other hand, the quality of coffee to free solar exposure is preferred by many consumers due to the quality and taste of the drink, and there are even researchers who in their processes have indicated that for some variables such

as production the system to free solar exposure it can be favored in productivity increases [12].

Other researchers such as [13] have conducted similar research to the identification of lustiness levels, but a study was conducted on 8 varieties of Arabica coffee in two provinces Quevedo and Gualea (Ecuador) from which, through the evaluation of the agronomic behavior of the varieties showed lustiness averages in scale 4 corresponding to the characteristic of plants with very good lustiness; situation similar to the present one that in most of them were presented in scale of lustiness 4.

Flowering: As shown in graph 2, the distribution of the flowering period was most marked in batch 2 with 40%, followed by 1 with 35% and finally the batches with the lowest representation were batch 3 and 4 with 10 and 5% in its corresponding order; it is clear that the stage of greatest representation was the E1 in all the lots, which justifies its representation in the graph; in the variable of variety the period of flowering (E1) was observed with greater representation in the caturra variety, and the values in the castle variety were less representative; and between production systems the direct sun exposure showed better results between the data obtained for each of the varieties under study.

It must be borne in mind that the flowering period is a periodic change directly associated with the productive stage of coffee cultivation [14], however, the evaluated flowering stage is not considered the main one; This corresponds to the evaluation carried out in the productive stage of the first semester of the year, which is responsible for the production distributions that are evident in the second semester as normally happens in the southern region of the country, specifically in the department of Cauca for this research thanks to the environmental and climatic conditions provided by the area [8].

The process of identification of flowering stages for the different batches evaluated showed scattered distributions in the lots and during the registration period (6 Months); the appearance of flower buds starts in lots 1 and 2; and in the middle of the follow-up process, buttons start to be identified in lot 4 and finally appearances of flower buds in lot 3; these sporadic distributions of little magnitude continue their development of coffee beans for productions of the second period that occurred at the end of the year in the southern region, as has been demonstrated by several investigations by the National Center for Coffee Research; besides the temperature conditions play a very important role in the flowering processes since they can inhibit or promote the flower inductions; for this specific investigation we have an average temperature of 25 ° C and 26 ° C and are close to those defined by [15] as the appropriate temperature to promote flower initiation.

Production: As can be seen in graph 3, the production percentages were represented as follows: with the highest percentage of production by number of grains in lot 4 (Castillo-sombra) with 37.2%, followed by lot 2 (Caturra-sol) with 30.2% and lot 3 (Castillo-sol) and 1 (Caturra-sombra) with16.5% and 15.9% in their corresponding order; this level of production in the varieties had greater representation in the Castillo variety with a percentage of 53.7% unlike the Caturra variety with 46.2%; these values confirm what was stated by Cenicafe 2011 where it determines that the castle variety is a variety composed of low size, slightly larger than caturra of long branches, large leaves, lustinessous, large grain, excellent cup quality, and production superior to that of the Caturra variety.

However, studies have been reported that show that the differences between the Castillo and Caturra variety are not as significant; it is the particular case of the research called: "Comparison of the quality of Nariño coffees according to variety"; which was disclosed at the Symposium of the American Specialty Coffee Association (SCAA) in Seattle, Washington, and indicating that coffees of these varieties grown and processed in similar conditions have very similar sensory characteristics, indicate that the castle can reach equal quality levels a caturra, however the difference that if taken into account is the resistance diseases where the caturra variety has presented resistance weaknesses against these characteristics [16].

Similar situation occurs in the productive quantity between the production systems, where the coffee agroforestry system presents a production of 53.1% and the system for free solar exposure presents percentages of 46.8%, thus considering again the benefits of coffee agroforestry systems with sustainable characteristics and contributing to the care of the environment; it must be taken into account that this can be determined by the agronomic management that is given to the crop, related to cultural work, density of planting, application of nutrients among others, since in different occasions and studies many varieties of free-ranging arabica solar have shown excellent productive results.

V. Conclusions

It concludes that the castle variety has a higher growth rate than the Caturra variety; however, this difference is not very significant; These conditions make it possible to verify what was stated in similar investigations where this characteristic had already been determined; and the behavior of the lustiness was better represented in the Caturra variety and between production systems the best lustiness is the production system under shade. That in relation to the flowering variable in productive stage this was presented in a dispersed manner during the monitoring period and its representation was more reflected in the Caturra variety in the two production systems corresponding to batch 1 and 2. In the process of production initially have better results in the Castillo variety and in the shade production system. It was found that lot 1 and 3 behave similarly to the standard behavior in cherry coffee production; now, batch 2 and 4 although they belong to a SP and different variety show an increase in production both in number and weight of grains. And in general, after making the relation between the number of grains and their weight, it was determined that they are directly proportional.

Acknowledgments

The research direction of the University Corporation Comfacauca-Unicomfacauca for supporting this process, the teacher Bibiana Montoya for his important advice, Supracafé for their support and spaces and each of the people who contributed to the realization of this work.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 4 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Effect of Mycorrhizal Inoculation (VAM) and Phosphorus Levels on Growth and Yield Attributes of Sunflower

By Kalaiyarasan C., M. V. Sriramachandrasekharan, S. Jawahar & R. Ramesh
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Abstract- The field experiment was conducted at Experimental Farm, Department of Agronomy, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu during July – October 2015 to study the effect of my corrhizal inoculation (VAM) and different levels of phosphorus on growth, yield attributes and yield of hybrid sunflower cv. Sunbred. The experiment was conducted by factorial randomized block design with two replications. The treatments of experiment consisted of 5 levels of P_2O_5 (0, 25, 50, 75 and 100kg/ha) applied in the presence or absence of VAM inoculates. The results revealed that growth, yield attributes and yield was significantly influenced by various P levels in the presence and absence of VAM inoculations. The growth and yield of sunflower was highest under mycorrhizal inoculated plants than non micorhizal inoculation. Among the various phosphorus levels tried, P_2O_5 at 100 kg ha-^{1} recorded maximum values for growth and yield, while P_2O_5 at 0kgha-^{1} registered minimum values for growth and yield of sunflower. Among the treatment combinations tried, mycorrhizal inoculation with P_2O_5 @ 100 kg ha-^{1} recorded maximum values for growth and yield attributes and yield of sunflower (2153 kgha-^{1}) but it was on par with M_2 P_3 (mycorrhizal inoculation with P_2O_5 @ 75 kg ha-^{1}). The lowest values of growth and yield attributes and yield were recorded by non-mycorrhizal inoculation with P_2O_5 @ 0 kg ha-^{1}.

Keywords: *sunflower, VAM growth and yield.*

GJSFR-D Classification: *FOR Code: 070302*

Strictly as per the compliance and regulations of:

Effect of Mycorrhizal Inoculation (VAM) and Phosphorus Levels on Growth and Yield Attributes of Sunflower

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Abstract- The field experiment was conducted at Experimental Farm, Department of Agronomy, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu during July – October 2015 to study the effect of my corrhizal inoculation (VAM) and different levels of phosphorus on growth, yield attributes and yield of hybrid sunflower cv. Sunbred.The experiment was conducted by factorial randomized block design with two replications. The treatments of experiment consisted of 5 levels of P_2O_5 (0,25,50,75 and 100kg/ha) applied in the presence or absence of VAM inoculates. The results revealed that growth, yield attributes and yield was significantly influenced by various P levels in the presence and absence of VAM inoculations. The growth and yield of sunflower was highest under mycorrhizal inoculated plants than non micorhizal inoculation. Among the various phosphorus levels tried, P_2O_5 at 100 kg ha^{-1} recorded maximum values for growth and yield, while P_2O_5at 0kgha^{-1} registered minimum values for growth and yield of sunflower. Among the treatment combinations tried, mycorrhizal inoculation with P_2O_5 @ 100 kg ha^{-1} recorded maximum values for growth and yield attributes and yield of sunflower (2153 kgha^{-1}) but it was on par with M_2 P_3 (mycorrhizal inoculation with P_2O_5 @ 75 kg ha^{-1}). The lowest values of growth and yield attributes and yield were recorded by non-mycorrhizal inoculation with P_2O_5 @ 0 kg ha^{-1}.

Keywords: sunflower, VAM growth and yield.

I. Introduction

Sunflower (*Helianthus Annuus* L.) belongs to the family Asteraceae, a new world plant, native of southern parts of United States of America and Mexico has been developed into a valuable source of edible oil and meal, with almost 20-27 percent protein and 40-47 percent oil (Saleem *et al*., 2003). It is easy to cultivate and grown in different conditions and soils. Sunflower oil has excellent nutritional properties, and has a relatively high concentration of linoleic acid (Seiler, 2007). It is also a wealthy source of vitamins A and D. The sunflower seed cake used for cattle feed which is a good source of protein (Gandhi *et al*., 2008). Indian soils are deficient in phosphorus. P is generally a limiting factor in sunflower growth and yield because P deficiencies reduce the accumulation of crop biomass (Zubillaga *etal*., 2002). P is an essential plant nutrient required for higher and sustained productivity of oil from sunflower. Its influence on seed yield, oil yield and oil quality has been well established (Bahl and Toor, 1999). Phosphorus is one of the most essential element for plant growth after nitrogen. It plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch and transporting of the genetic traits. A great advantage of feeding the plants with phosphorus is to create deeper and more abundant roots. Phosphorus causes early ripening in plants, decreating grain moisture, and improving crop quality. However, the availability of this nutrient for plants is limited by different reactions. A great proportion of phosphors in chemical fertilizer becomes unavailable to the plants after its application in the soil and further, the mobility of this element is very slow in the soil and cannot respond to its rapid uptake by plants. This causes the creation and development of phosphorus depleted zones near the contact area of roots and soil in rhizosphere. Therefore, the depletion zones and helps to absorb the phosphorus from a wider area by developing an external network around root system. Mycorrhiza hassymbiotic association between the soil fungi and roots of higher plants (Smith *et al*., 2010). These fungi enhance the plant growth through making availability of mineral nutrients such as P, Zn and Cu (Phiri *et al*., 2003). Colonization of AM fungi in cortical tissues of sunflower increased the growth parameters of sunflower (Jalaluddin and Hamid, 2011).

The current trend is to explore the possibility of supplementing chemical fertilizers with organic ones, more particularly biofertilizers of microbial origin. In this context, VAM fungi are receiving greater attention in their beneficial effects on plant growth. Vesicular-arbuscular mycorrhizae (VAM) are widespread in soils, and often the growth of mycorrhizal plants will be higher in comparison to non-mycorrhizal plants. This beneficial effect on plant growth has largely been attributed to higher phosphorus (P) uptake and consequently better P nutrition of mycorrhizal plants (Antunes *et al*. (2007). The beneficial effects of VAM inoculation on P uptake, growth and yield of sunflower have not been carried out. Hence, the present study was taken up to find out the

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effect of mycorrhizal inoculation andphosphorus on the growth and yield attributes of hybrid sunflower.

II. Materials and Methods

Field experiment was conducted during July – October 2015 at the Experimental Farm, Department of Agronomy, Faculty of Agriculture, Annamalai University. The experimental soil was clay loam with pH 8.1, OC 5.0g kg^{-1}, available N (235 kg ha^{-1}), P (22.1 kg ha^{-1}) and K (356 kg ha^{-1}). The experiment consisted of ten treatments and was laid out in factorial randomized block design with two replications. The treatments imposed in the experiment are M_1-Non inoculated and M_2-inoculated *Glomus intraradices* were tried along with different phosphorus levels (P_0-0, P_1-25, P_2-50, P_3-75 and P_4-100 kg P_2O_5 ha^{-1}) through SSP. Recommended dose of 60:60 kg of N&K ha^{-1} was applied in the form of Urea and MOP respectively. Half the dose of N and entire dose of K were applied basally. The remaining quantity of N was applied at 30 DAS. P was applied as per treatment schedule. The mycorrhizal inoculum was applied near the root zone of sunflower. 2gm VAM was applied per plant by placement method. At harvest, plant height was recorded from the first node at the bottom of the plants to the bottom of the head and expressed in cm. The leaf area index (LAI) was calculated by dividing the total leaf area of the plant by the land area occupied. To estimate dry matter production (DMP) the selected plant samples were collected washed, air dried and kept in an oven at 80°C till constant weight was obtained and expressed in Kg ha^{-1}.The yield parameters and yield were recorded at harvesting stage of plant. The head samples for yield were also dried to constant weight and threshed mechanically. Seed yield was adjusted to a 10% moisture basis. Filled seeds and empty hulls were separated by hand. Hereafter, grain number head-1 refers to filled grains only. Data collected were subjected to statistical analysis of variance according to Gomez and Gomez (1989).

III. Results and Discussion

a) Growth Attributes

Mycorrhizal inoculated plants significantly influenced the growth attributes *viz.,* plant height, LAI, DMP and CCI (Table 1). Mycorrhizal inoculation recorded the highest plant height at harvest (147.0 cm), leaf area index at flowering (4.26), dry matter production at harvest (4994 Kg ha^{-1}) and chlorophyll content index (23.43) at flowering stagethan non - mycorrhizal inoculation. This might be due to the formation of external mycelium around the roots by AM fungi which possibly helped to increase the availability of nutrients to the surface of the roots and thereby increased the nutrient uptake andgrowth of the plant. Similar finding was earlier reported by Kavitha and Nelson (2014).

Table 1: Effect of Mycorrhizal Inoculation (VAM) and Phosphorus Levels on Growth and Yield Attributes and Yield of Sunflower

Treatments	Plant Height (cm) at Harvest	LAI at Flowering	DMP at Harvest (kg ha^{-1})	Chlorophyll Content Index	Head Diameter (cm)	Number of Filled Seeds $Head^{-1}$	100 Seed Weight (g)	Seed Yield (kg ha^{-1})	Stalk Yield (kg ha^{-1})
VAM									
M_1	133.1	3.65	4485	20.39	15.7	514	5.31	1438	3570
M_2	147.0	3.91	4994	23.43	17.7	660	5.83	1845	4069
SEd	1.09	0.016	25.11	0.19	0.09	8.02	0.016	28.46	20.75
CD(P=0.05)	2.34	0.034	53.86	0.41	0.20	17.19	0.034	61.05	44.51
Phosphorus Levels(kg ha^{-1})									
P_0	98.3	2.91	3645	16.21	11.12	321	4.81	973	2692
P_1	126.0	3.48	4101	18.49	14.4	428	5.02	1197	3179
P_2	134.1	3.67	4491	21.03	15.8	528	5.39	1477	3589
P_3	146.3	3.92	5051	23.38	17.8	661	5.81	1843	4129
P_4	153.8	4.05	5316	24.73	18.9	730	6.06	2048	4379
SEd	1.54	0.023	35.51	0.27	0.13	11.34	0.022	40.25	29.35
CD(P=0.05)	3.30	0.049	76.18	0.58	0.29	24.32	0.048	86.34	62.95

Among the different levels of phosphorus, application of P2O5 at 100 kg ha-1 significantly recorded highest plant height at harvest (153.8 cm), leaf area index at flowering (4.05), dry matter production at harvest (5316 Kg ha^{-1}) and chlorophyll content index (24.73) at flowering stage. This might be attributed to the P stimulating effect on root growth and expansion by increasing crop growth rate. The lowest values for growth attributes were recorded in the treatment which did not receive phosphorus.Similar findings were earlier reported by Adebayo *et al*. (2010) and Abubaker Ali *et al.* (2014).

The interaction effect between the mycorrhizal inoculation and phosphorus was significant (Table 2). The treatment combination of mycorrhizal inoculation along with P_2O_5 at 100 kg ha^{-1} recorded maximum values for growth attributes, but it was on par with mycorrhizal inoculation along with P_2O_5 at 75 kg ha^{-1}. The highest values under these treatments might be due to mycorrhizal inoculation, because this bio fertilizer can enhance absorption of phosphorus by plant. The lowest growth attributes was recorded in the treatment combination of non mycorrhizal inoculation with 0 kg P_2O_5 ha^{-1}. This could be due to inadequate availability of nutrients. This result is in conformity with the findings of Khirood Doley and Paramjit Kaur Jite (2012).

Table 2: Interaction Effect between VAM and Phosphorus on Growth and Yield Attributes of Sunflower

Treatments	Plant Height (cm) at Harvest	LAI at Flowering	DMP (kg ha^{-1}) at Harvest	Head Diameter (cm)	No. of Filled Seeds Head^{-1}	Test Weight (g)	Seed Yield (Kg ha^{-1})	Stalk Yield (Kg ha^{-1})
M_1P_0	98	3.11	3521	11.1	298.1	4.31	880	2712
M_1P_1	121	3.58	3883	13.6	366.1	4.78	1019	2946
M_1P_2	126	3.78	4175	14.4	435.1	5.07	1219	3271
M_1P_3	137	4.04	4697	16.5	563.1	5.47	1571	3795
M_1P_4	149	4.36	5184	18.4	692.4	5.94	1942	4268
M_2P_0	109	3.24	3689	11.9	328.6	4.69	996	2968
M_2P_1	131	3.90	4319	15.2	490.6	5.26	1376	3412
M_2P_2	142	4.15	4807	17.2	621.8	5.71	1735	3908
M_2P_3	156	4.48	5403	19.1	758.4	6.16	2114	4464
M_2P_4	159	4.50	5448	19.3	768.2	6.18	2153	4491
SEd	2.18	0.035	50.22	0.19	16.03	0.032	56.9	41.5
CD (P = 0.05)	4.65	0.075	107.73	0.40	34.39	0.068	122.1	89.03

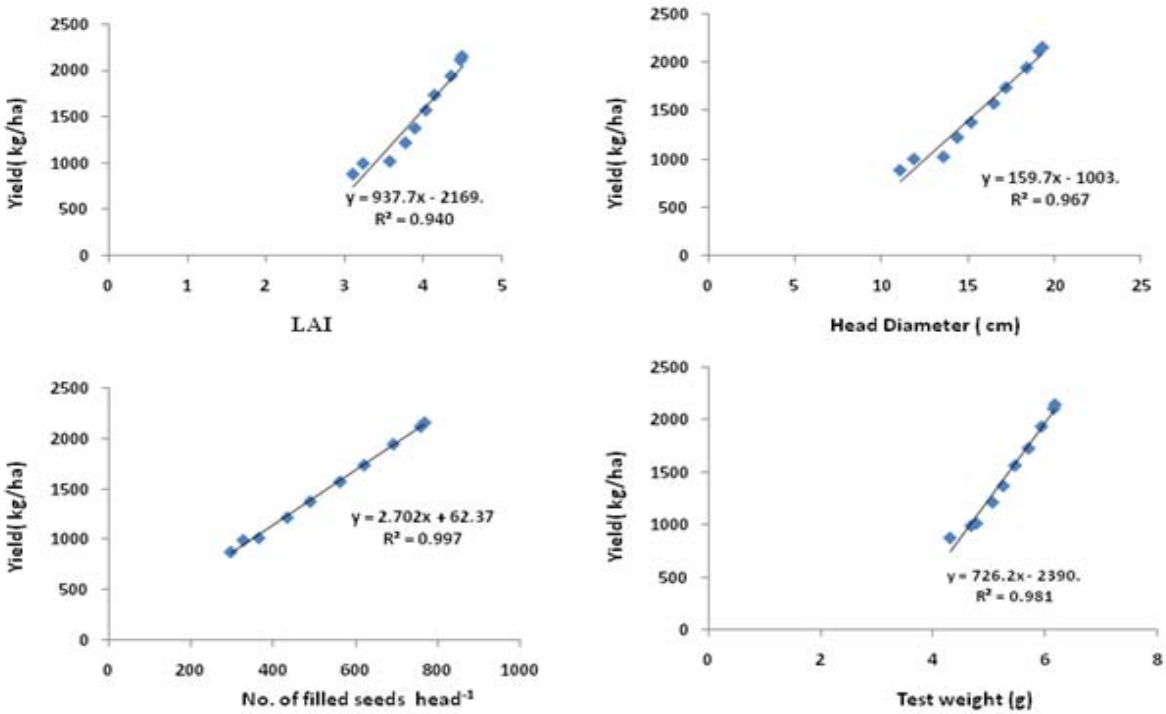

Fig. 1: Linear Relationship between Seed Yield with a) LAI, b) Head Diameter, c) No. of Filled Seeds / Head, d) Test Weight

b) Yield Attributes and Yield

Mycorrhizal inoculated plants showed significant influence on yield attributes and yield (Table 1). Mycorrhizal inoculation recorded the maximum head diameter (17.7cm), number of filled seeds head-1 (660), 100 seed weight (5.83 g) and seed yield (1845 kg ha-1) and stalk yield (4069 kg ha-1) than non-mycorrhizal inoculation. The per cent increase in seed and stalk yield due to mycorrhizal inoculation was 28.3 and 13.9 over non-mycorrhizal. Many researchers suggested that VAM symbiosis increased the units of photosynthesis, and so as to increase the rate of photosynthetic storage and export at the same time (Auge, 2001).

Phosphorus levels significantly influenced the yield attributes and yield (Table 1). Among the different levels, P_2O_5 at 100 kg ha^{-1} produced maximum head diameter (18.9 cm) number of filled seeds $head^{-1}$ (730), 100 seed weight (6.06 g) and seed yield (2048 kg ha^{-1}) and stalk yield (4379 kg ha^{-1}). The best treatment caused 53 and 38 per cent increase in seed and stalk yield over control. The lowest value for yield attributes and yield was recorded in the treatment P_2O_5 at 0 kg ha^{-1}. This might be due to the role of phosphorus in cell division and cell enlargement, photosynthesis, which ultimately affect the yield attributes. Similar finding was earlier reported by Ghazanfar Ullah Sadozai (2013).

The interaction effect between the mycorrhizal inoculation and phosphorus was not significant (Table 2). The treatment combination of mycorrhizal inoculation along with P_2O_5 at 100 kg ha^{-1} recorded higher values for yield attributes and yield but it was on par with mycorrhizal inoculation along with P_2O_5 at 75 kg ha^{-1}. The mycorrhizal inoculation caused a saving of 25 kg P_2O_5/ha. This might be due to more availability of phosphorus and other nutrients at both vegetative and reproductive stages. Similar findings were earlier reported by Hossein Soleimanzadeh (2012) and Khirood Doley and Paramjit Kaur Jite (2012). The lowest values of yield attributes and yield were recorded under the treatment combination of non mycorrhizal inoculation with 0 kg P_2O_5 ha^{-1}.This might be due to the absence of mycorrhiza resulted in reduced growth and yield attributing characters and seed and stalk yield. The effect due to different treatments on yield was confirmed by significant positive linear relationship noticed between seed yield with LAI, Head diameter, number of filled seeds per head and test weight (Fig. 1). The present result was in harmony with earlier reported by Ultra Jr *et al*. (2007) and Mostafa Heidari and Vahid Karami (2014).

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 4 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

An Automatic Farmland Irrigation System for Northern Ghana

By Enoch Tetteh Amoatey & Henry Kwame Atiglah
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Abstract- Agriculture plays a very important role in Ghana and Africa at large. It's a source of income and sustenance for the large majority of Ghanaians. Recently, there have been huge drawbacks in the development of the agricultural sector in the country due to incidents of drought most profoundly in the northern region, which has led to the deterioration of several farmlands. This has directly and indirectly affected the lives of many, most especially those who depend on it for a living. This project is thus developed with the main objective of helping to address the issues of crop destruction due to the inability of farmers to store water in the rainy season and use it later on when there is not much water or lack of rainfall. The proposed system would address this challenge by having a water tank, which would store the water to be used for irrigation. The system would then have a soil moisture sensor in the soil, to measure the amount of water in the soil. If the water in the soil falls below a preset threshold for the farmland, the system would automatically irrigate that portion of the farm to keep the soil in good condition. This system is achieved by the use of a PIC16F887A microcontroller, which is connected to the soil moisture sensor, which takes the readings for the microcontroller. The microcontroller upon receiving the soil moisture readings from the soil moisture sensor would display the readings on an LCD and if the soil is in good condition the system performs no action, but if the soil is dry, the water pump is activated to pump water for the purposes of irrigating the soil.

Keywords: *water tank, soil moisture sensor, microcontroller, irrigation.*

GJSFR-D Classification: *FOR Code: 960103*

Strictly as per the compliance and regulations of:

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I. Introduction

In the fast-paced world, human beings have resolved to be living much easier by incorporating automated systems in their day to day activities. The continuous increase in demand of food is leading to the development of food production technologies to increase food supply and availability. In the world of advanced electronics, the life of human beings should be more convenient, thus the need to develop an automated form of an irrigation system. It is a simple and precise system for farmlands with no close reach to the water supply. It also helps in saving time and reducing the manual labor required in agriculture, hence those resources such as human effort and time can be put into other areas, thus increasing productivity and eventually maximizing the net profit of farmers. The main reason, which calls for such technological systems, is the lack of proper irrigation on farmlands. Irrigation is the artificial application of water to the soil or crops to help growth, typically by means of channels or water reservoir. In the present era, farmers have been using the conventional irrigation methods which involve irrigation overhead through manually controlled sprinklers, flood type feeding systems and farmers making small gutters from river banks to their farm. This system is good but for the fact that some irrigation is being done on timely basis and also when the farmer is available, while some amount of water entering the farmland can't be regulated, this leads to the plants or soil having too much water at a particular time or less than required. Thereby, not facilitating the growth of plants and also not improving agricultural production. An automated system, on the other hand, would be measuring the amount of water present in the soil and would irrigate when required. This would make it a lot easier for the farmer to irrigate more lands and increase crop yield without putting in as much effort as would be required by the conventional system currently being used.

II. Block Diagram And Description

The block diagram of the system is shown in figure 2. The system has a power supply, which consists of a voltage regulator used to stabilize the current flowing into the circuit. The output voltage of the power supply system is 5 volts as that's what is required for the microcontroller. The PIC16f887A microcontroller acts as the brain of the system. The soil moisture sensor, which measures the amount of water present in the soil, sends this data to the comparator, which compares this data with a pre-defined value to check if the amount of water in the soil is below or above the permissible value. The output of the comparator is then sent to the microcontroller. The water level indicator indicates the level of water in the tank from which water is pumped into the soil. The water level sensor sends the level of the water to the microcontroller. The microcontroller then processes these received data from the water level indicator and also for the amount of water in the soil. It then turns ON a number of LEDs to indicate the level of the water. A relay is attached to the microcontroller which is activated to pump water into the soil if there is not enough water in the soil and there is water in the water tank. A liquid crystal display is connected to the microcontroller, which displays all ongoing processes of

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the system and also displays the status of the various readings from the soil moisture sensor and the water level indicator.

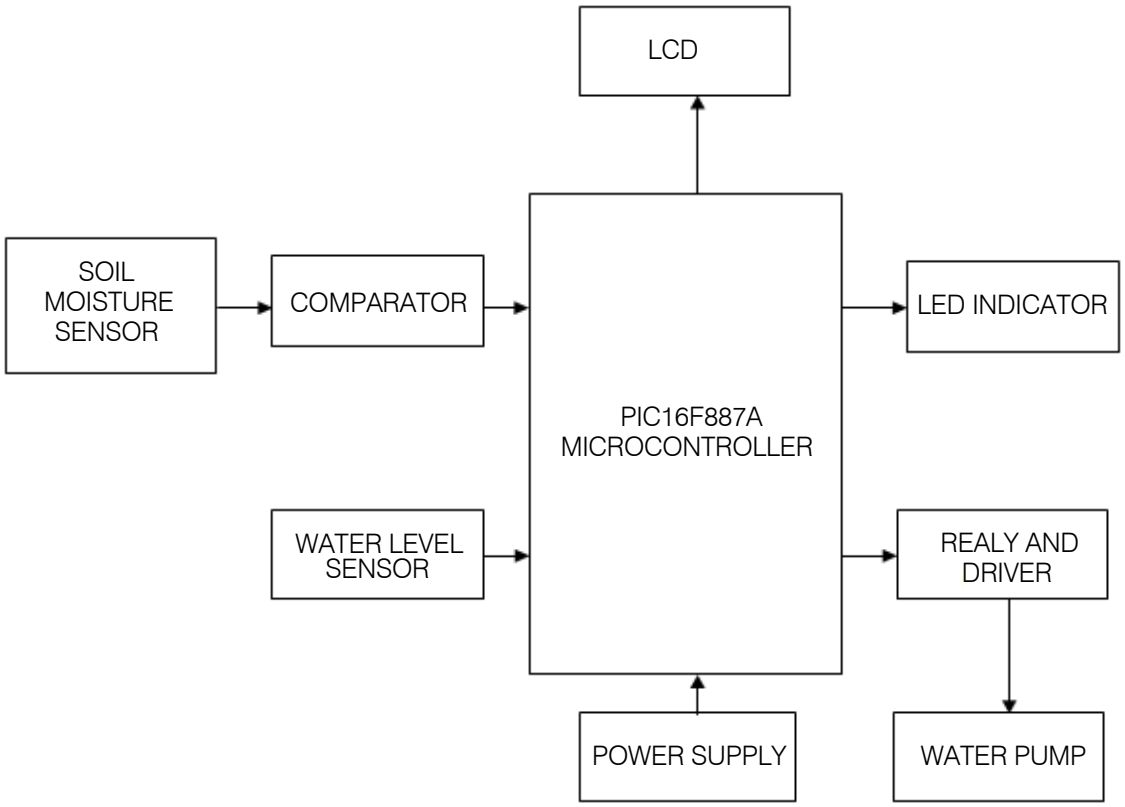

Fig. 1: Block Diagram of the System

III. Circuit Diagram And Operation

There is an LM7805 voltage regulator connected to two 100uf capacitors which serve as the power supply of the system. They regulate the voltage flowing into the system to a fixed 5v since almost all the components of the circuit require 5V to operate. The voltage from the voltage regulator then goes to power the microcontroller, LCD and other components in the circuit. There is a PIC16f887A microcontroller which controls all the activities of the system. It has a crystal oscillator with the appropriate load capacitors to drive the microcontroller to a specified frequency. An LCD is connected to the microcontroller to display the readings of the sensors and also display any operation of the system. An LM393 op-amp is used as a comparator to compare the reading of the soil moisture sensor with that of a predefined value, hence the reading of the soil moisture sensor is first sent to the comparator microcontroller. A reset button is attached to the microcontroller in order to pull its reset pin low to reset the microcontroller then the output of the comparator is sent to the There is also a water level indicator, whose float switches are placed at certain levels in the tank. This conducts when water reaches those levels, and the appropriate signal is sent to the microcontroller. The microcontroller then sends a high signal to turn ON the corresponding LED to indicate the level of the water in the tank. The LEDs have a limiting resistor each of 220 Ohms to limit the amount of current flowing through them. A ULN2003 Darlington transistor IC is placed in the system to serve as a driving transistor for the relay so only one transistor in the array is used. A 12V relay is then connected to the transistor IC which has a water pump connected to it. The relay activates the water pump to begin the pumping process if the water in the soil is below the required level and also if there is enough water in the water tank. A LED and its limiting resistor of 220 Ohms is placed in parallel with the water pump to indicate the ON and OFF status of the water pump

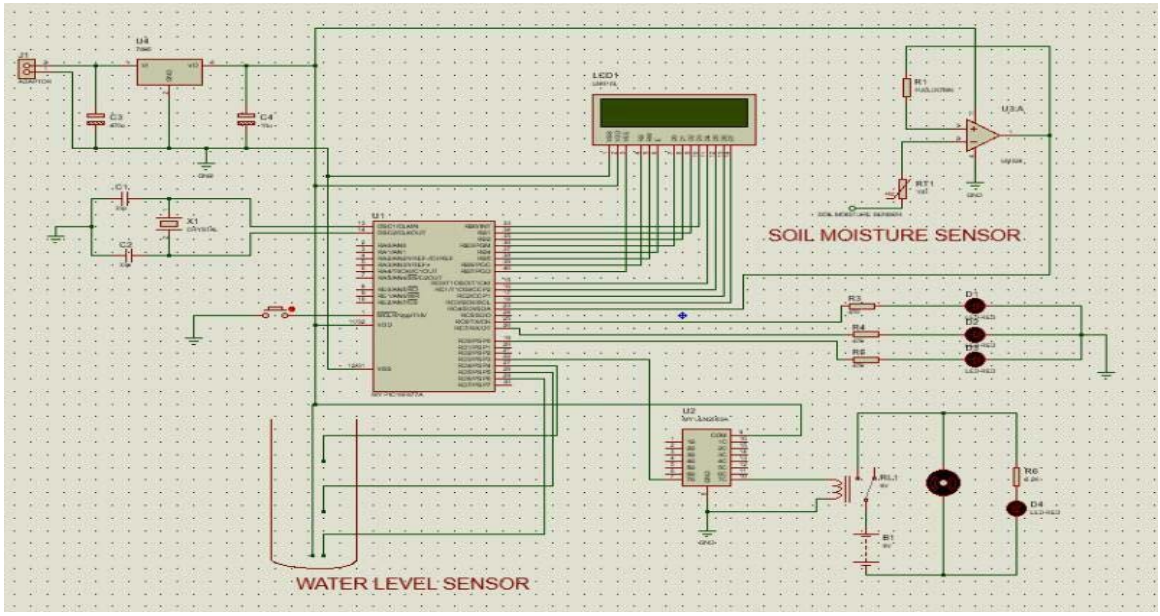

Fig. 2: Circuit Diagram of the System

IV. Results And Discussion

The project was successfully designed and constructed after which we performed some experiments in order to make some observations. The device was tested on the 2nd and 4th of April 2018 on the Tamale Technical University Campus. Both tests produced the expected results which were as follows. It should be noted that there was no rain for some days before the experiment was performed on the 2nd of April whereas there was rain on the 4th of April 2018.

Table 1: Observations for 2nd April 2018

Condition	Status
Soil is dry and tank is empty	Water pump on
Soil is dry and tank is full	Water pump off
Soil is wet and tank contains water	Water pump off

2nd April 2018

Table 2: Observations for 4th April 2018

Condition	Status
Soil is wet and tank is full	Water pump off
Soil is wet and tank is empty	Water pump off

4th April 2018

Thus, it was realized that on the 2nd of April because there was no rain several days before this experiment was conducted the soil was dry hence, there was the need for it to be irrigated. The water pump however did not operate when there was no water in the tank but remained off. The water tank however began to pump water to irrigate the soil once there was enough water in the water tank. After irrigating the soil for some time thought there was enough water in the tank, the water pump was deactivated and this was because the soil now had enough water after a period of irrigation.

On the 4th of April however, there was enough water in the soil from the rain, which had fallen earlier on. Hence, there was no need to irrigate the soil. It could then be observed that for both instances where there was water in the tank and when there was no water in the tank the water pump still remained off to indicate that the soil did not require any water to irrigate it. The system thus worked just as we expected.

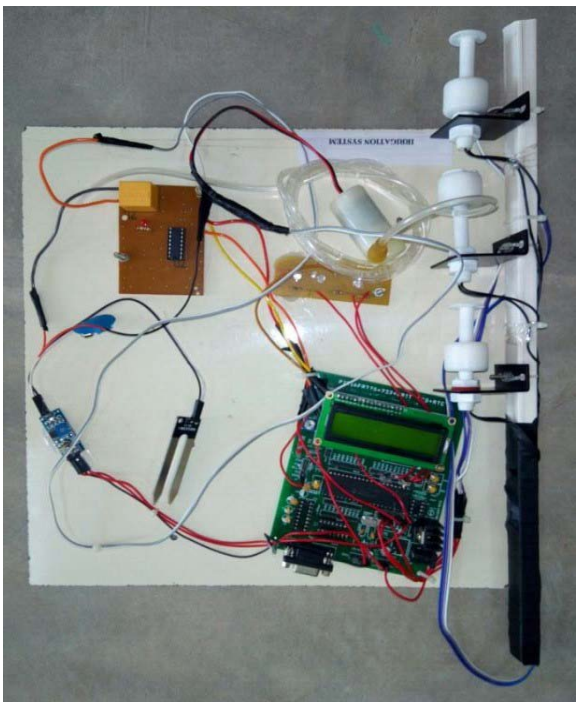

Fig. 3: Diagram of Complete System

V. Conclusion

The design and implementation of an automatic irrigation system using soil moisture and water level indicator was successfully carried out. The objective to measure the water level in an overhead tank and irrigate the soil efficiently was successfully achieved. In the process of testing the system, it was observed that the soil moisture was measured and thus the system was able to efficiently measure the amount of water in the soil. Only that there were some challenges in the design of the hardware especially when we worked with the float switch as it could not measure the water level continuously but only in steps. Thus, the water level could be determined only for specific levels. Continuous testing also showed that the station would not be effective if the hardware parts of the stations are not of higher standards. The automatic irrigation system using soil moisture and water level indicator can be deployed in the conventional open farming systems as well as in advanced agricultural systems such as greenhouses.

VI. Future Scope

In future expansions of this project, the automatic irrigation system using soil moisture and water level indicator could have an option to connect to the internet so that the system can be controlled by the user for other specific purposes and for the user to be able to monitor the real-time status of the farm soil. The measured data of the soil should also be stored to allow for analysis of those data for better understanding of the system and solving data analysis problems. Also, higher standards of components parts should be used so that when the station is installed it can withstand adverse conditions.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 4 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Integrated Management against Seed-Borne Diseases of Farmers Stored Chickpea

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Abstract- Performance of different treatments was evaluated to find out effective control measures against some fearsome diseases of farmers stored Chickpea seeds. A set of seven treatments were used in the study: two fungicides (Provax 200WP and bavistin 70WP), two botanical extracts (Neem leaf and Garlic clove), bio-fertilizer, breeder seeds and healthy seeds. All treated seeds including breeder seeds and healthy seeds performed better compared to control. Fungicides successfully inhibited fungal growth in laboratory and field conditions. In blotter method, seeds treated with Provax 200WP and Bavistin 70WP showed the highest results against Fusarium oxysporum, Sclerotium rolfsii and Botrytis cinerea compared to control and all other treatments. In controlling the radial growth of fungi at 100, 200 and 400ppm, Provax 200WP performed the best against Sclerotium rolfsii, Bavistin 70WP against Fusarium oxysporum and both of the treatments performed best against Botrytis cinerea and % inhibition increased with increasing concentrations. Also, the highest plant population and the lowest incidence per pot or plot exhibited with fungicides application, and in some cases, a combination of healthy seeds and Bavistin 70WP showed insignificant results.

Keywords: *integrated, chickpea, disease, fungicides, botanical, bio-fertilizer, healthy seeds.*

GJSFR-D Classification: *FOR Code: 060704*

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Abstract- Performance of different treatments was evaluated to find out effective control measures against some fearsome diseases of farmers stored Chickpea seeds. A set of seven treatments were used in the study: two fungicides (Provax 200WP and bavistin 70WP), two botanical extracts (Neem leaf and Garlic clove), bio-fertilizer, breeder seeds and healthy seeds. All treated seeds including breeder seeds and healthy seeds performed better compared to control. Fungicides successfully inhibited fungal growth in laboratory and field conditions. In blotter method, seeds treated with Provax 200WP and Bavistin 70WP showed the highest results against *Fusarium oxysporum, Sclerotium rolfsii* and *Botrytis cinerea* compared to control and all other treatments. In controlling the radial growth of fungi at 100, 200 and 400ppm, Provax 200WP performed the best against *Sclerotium rolfsii,* Bavistin 70WP against *Fusarium oxysporum* and both of the treatments performed best against *Botrytis cinerea* and % inhibition increased with increasing concentrations. Also, the highest plant population and the lowest incidence per pot or plot exhibited with fungicides application, and in some cases, a combination of healthy seeds and Bavistin 70WP showed insignificant results. Combination of botanicals, bio-agents and healthy seeds with fungicides may be considered as part of the integrated approach. Therefore, application of fungicides could be suggested as the best treatment to control storage and field diseases of farmers stored chickpea seeds.

Keywords: integrated, chickpea, disease, fungicides, botanical, bio-fertilizer, healthy seeds.

I. Introduction

Chickpea (*Cicer arietinum* L.), is an annual pulse crop and mostly appreciable for its high edible protein content (20.8%) (Saxena and Singh, 1987). Pulses crops are inherently low yield potential, susceptibility to poor seed quality, diseases and insect pests and sensitivity to microclimate changes, contribute to their yield instability (Fakir and Rahman, 1989). A pure viable seed of a high yielding variety is of little or no use, if that seed is infected or contaminated by pathogens (Rahman *et al*., 1982). Most of the farmers are bound to use their stored seeds because only 1.15% seeds are produced by BADC. In most of the cases farmers stored seeds are badly infested with stored grain pests and molds resulting very poor germination (Mia *et al*., 2000, Khokon *et al*., 2005). More than 60 pathogenic fungi including 20 major ones can infect seeds and transmit different diseases in the field causing considerable yield loss (Bakr and Rashid, 2007). Seed-borne diseases are the major limitations for chickpea cultivation. Out of 17 diseases of chickpea recorded so far in Bangladesh, Botrytis Gray Mold is as one of the most damaging diseases of chickpea (Bakr, 1994). The fungus has been reported to cause up to 80% yield losses. The wilt disease (*Fusarium oxysporum)* and Collar rot (*Sclerotium rolfsii)* are other destructive diseases (Nene *et al*., 1996). The pathogens are soil, seed and air borne and can also survive in the residual stubbles for more than three years (Nene *et al*., 1987). The pathogens may remain alive in the seed after harvest and in storage in dormant condition.

Proper seed health technology such as seed cleaning, integrated seed disease management approach and field seed health standard is needed for the improvement of quality farmer stored chickpea seeds. According to the experts, 20% crop yield can be increased by using quality seed (Faruque, 2006). Still, research on improvement of farmer's stored chickpea is scanty. Maintaining the quality of farmer's seeds is essential as to ensure increased chickpea production. Therefore, this present study was undertaken to evaluate the efficacy of integrated measures to find out appropriate sole or combine control strategies for farmers stored chickpea diseases.

II. Materials and Methods

a) Collection of Seed Samples

The variety BARI Chola-5 of Chickpea (*Cicer arietinum* L.) was used in the study as a test crop. The farmer's seed was collected from 16 farmers of Godagari, Rajshahi district through focal group discussion (FGD). Each farmer used their own stored seeds for sowing of BARI Chola-5 and had at least 0.135 ha of his chickpea cultivable field. The seed samples (1 kg/sample) were collected for the present studies following the International Rules for Seed Testing (ISTA, 2001) at 15 days before sowing. The seed samples were preserved at cool room temperature in the laboratory of plant pathology department of Bangabandhu Sheikh Mujibur Rahman Agricultural University for subsequent use in the study. The studies

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were carried out in the Plant Pathology laboratory, Bangladesh Agricultural Research Institute (BARI), Gazipur.

b) Isolation and Identification of Fungi

The samples of infected plant parts were collected from characteristic field symptoms of foot and root rot (*Sclerotium rolfsii*), Fusarium wilt (*Fusarium oxysporum*) and Botrytis Gray Mold (*Botrytis cinerea*). The plant samples were cut into small pieces for surface sterilization (1% HCl) and parts were placed in PDA media to allow growth of fungi as to prepare pure culture. The fungi were identified based on morphological characteristic of fungi as described by Mathur and Kongsdal (2003).

c) Mortality of Seedling (Pot Experiment)

To determine the effect of seed treatments on seedling mortality an experiment was set in the pot (12 X 9.5 inch). The experiment was laid out in Complete Randomized Design (CRD). Pots were filled with mixture of sand and soil. Then the pots were inoculated by *Sclerotium rolfsii*. 25 seeds were sown in each pot and four pots were used as one replication for each sample.

d) Preparation of Mixture of PDA Media with Treatments

Two chemical viz., Provax 200 WP and Bavistin 70 WP at 100, 200 and 400 ppm and two plant extract viz., Neem and Garlic at 5, 10 and 20% concentration were used to determine the effect of fungicides on radial growth of fungus. PDA was prepared by mixing infusion of 200g peeled potato, 20g dextrose and 17g agar in 1000 ml distilled water. The medium was cooked properly and poured into conical flasks at 100 ml per flask. Before solidification, requisite quantity of individual fungicides (Provax 200 and Bavistin 70 WP) was added to the medium to have concentrations of 100, 200, 400 ppm and botanical extracts (Garlic and Neem) was added to the medium to have concentrations of 5, 10 and 20%. After thorough mixing the medium was autoclaved at 121° C under 1.1 kg/cm^2 pressures for 20 minutes. Approximately 15ml of melted PDA mixed was poured into each 90 mm petri dishes.

e) Determination of Radial Growth Inhibition

The effect of fungicides on radial growth of *Botrytis, Fusarium and Sclerotium* were determined on the prepared PDA medium. The 5mm discs of 3 days old PDA cultures of fungi were used to inoculate the media. The discs were cut with a flame sterilized cork borer (5mm diameter). The inoculums were placed at the center of the test plates using a flame-sterilized needle at one disc per plate inside a clean bench. Three plates were used for each dose of every fungicide. Three replicated PDA plates received no fungicides were also inoculated as the control. The inoculated plates were incubated at 27^0 C and data on radial growth was taken after 60hrs of inoculation. The diameter of the colonies on PDA with and without fungicides were measured from the bottom side of the petri dishes. Inhibition of radial growth was computed based on colony diameter on control plate using the following formula shown below:

$$\% \text{ Inhibition} = \frac{X - Y}{X} X\ 100$$

X = radial growth of control plates, and Y = radial growth of fungicide-treated plates

f) Evaluating performance of different treatments against fungi in blotter paper and field conditions

The experiments were conducted in the Pulse Research Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Eight treatments with four replications were used in the experiment namely T1 = Provax-200 WP (0.25%) + Spraying Provax-200 WP (0.1%), T2 = Bavistin-70WP (0.25%) + Spraying Bavistin-70 WP @ (0.1%), T3 = Neem leaf extract (1:1) + Spraying neem leaf extract @ 1: 9 of water, T4 = Garlic clove extract (1:1) + Spraying garlic clove @ 1:9 of water, T_5 = Apparently healthy seeds + Spraying Bavistin-70 WP @ 1ml/l water, T_6 = Bio-fertilizer + Spraying Bavistin-70 WP @ 1ml/l water, T7 = Breeder seed + Spraying Bavistin-70 WP @ 1ml/l water and T_8 = Control (farmer's seed). Seeds were taken in a plastic container (9cm) and required amount of fungicides were added. The container was shaken well for uniform coating on to the seeds. After 24 hours the treated seeds were used for studying the efficacy of the fungicides. Two plant extract i.e., Neem leaf (*Azadirachta indica*) and Garlic (*Allium sativum*) @ 1:1 (water: extracts) concentrations were used for the experiment. Two different plant species were collected from the campus of BARI, Gazipur that was used in this study. The collected plant parts were chopped after cleaning under running tap water. The extracts were prepared by crushing the plant parts in a blender with distilled water at 1:1 (100g crushed plant materials in 100 ml water). However, extracts were used for field treatment as spraying purpose then it's ratio was 1: 9 (100g crushed plant materials in 900 ml water). The extracts were filtered through cheesecloth. The extracts thus obtained were kept in a refrigerator at 4±1° C until use. The seeds were dipped into 1:1 dilution of Garlic and Neem leaf extracts for 20-30 minutes. After proper covering of the seed coat with the extracts and seeds were used for studying the efficacy of the applied botanicals. Four hundred seeds were taken in a beaker (500 ml) and 4 drops of water were added for moistening the seed surface uniformly to allow maximum adherence of the Bio-fertilizer on the whole surface of seeds. Seeds were then treated with Bio-fertilizer @ 4% of seed weight until the whole surface of seeds was coated with the Bio-fertilizer. Seeds were taken randomly and tested following the blotter method to test the efficacy of fungicides and botanicals. The experiment was laid out

in Completely Randomized Design (CRD) with four replications. Seed-borne infection of fungi was observed at the 8th day of incubation.

For field evaluation, treated seeds were sown in lines about 2.0 cm depth after air drying and immediately covered with soil. The line to line and plant to plant distances were 30 cm and 5 cm respectively. The seeds were sown in the field in the afternoon at the rate was 50 kg/ha. Fungicides and botanicals were sprayed in the same chemicals and botanicals which were described previously. Fungicides and botanicals were spayed 3 times at 10 days of intervals in the experimental plot to reduce the major seed-borne diseases of Chickpea. The sprays were started at flowering stage of Chickpea plants. The field experiment was conducted in a Randomized Complete Block Design (RCBD) having three replications for each treatment. Each unit plot size was 4m × 3m = 12m2. The collected data were analyzed by ANOVA. The mean differences among the treatments were compared by Duncan's Multiple Range Test (DMRT). A statistical computer package MSTATC was used for analyzing the data.

III. Results

a) *Seed treatment in blotter method for controlling seed-borne fungi*

Provax 200 WP showed the highest efficacy to control *Aspergillus niger* over untreated seeds followed by Bavistin 70 WP and Bio-fertilizer. Untreated seeds showed the highest percentage of infection (81.00 %) and the lowest (14.75%) was recorded in Provax 200WP treated seeds. All the treated seed appeared to be fruitful to combat *Aspergillus flavus* in comparison with the untreated control seeds. The maximum infection (12.25%) was found in control, and the lowest (1.75%) was recorded in Bavistin 70WP treated chickpea seeds. In suppressing the growth of *Fusarium sp.,* all the treated seed appeared to be effective in comparison with the control treatment. The highest incidence (42.25%) was found in untreated seeds, and the minimum (14.55%) of *Fusarium* spp. was in Provax 200WP treated seeds and was followed by Bio-fertilizer and Bavistin 70 WP treated seeds. Maximum (10.25%) *Sclerotium rolfsii* was recorded in untreated seeds, and the minimum (0.50%) was in Provax 200WP treated seeds.

Alternaria spp. were found to be inhibited by all seed-treating agents. The highest prevalence (16.50%) of *Alternaria spp*. were in untreated control seeds, and the minimum (2.00%) was in Bio-fertilizer treated seeds and healthy seeds. All seed treating chemicals and plant extract inhibited *Penicillium notatum* compared to that of control. The maximum occurrence of seed-borne infection by *Penicillium notatum* (63.00%) was recorded in untreated seeds, while Provax 200WP (6.25%) and Bavistin 70WP (2.50%) treated seeds showed the lowest infection. The highest incidence (45.25%) of *Rhizopus stolonifer* was in untreated seeds, and the minimum (12.00%) was obtained in neem leaf extract followed by Provax 200WP (15.00%) treated seeds. *Botrytis cinerea* was found to be controlled by most of the treatment used. The highest seed infection (11.25%) by *Botrytis cinerea* was recorded in control, and the minimum incidence (0.75%) in Bavistin 70WP treated seeds followed by Provax 200WP (1.25%) treated seeds and utilizing healthy seeds (1.50%) (Table 1).

Table 1: Seed Treatments in Controlling Prevalence of Seed-Borne Fungi of Farmers' Stored Chickpea Seeds

Treatment	Aspergillus Niger	Aspergillus Flavus	Fusarium Spp.	Sclerotium Rolfsii	Alternaria Spp.	Penicillium Spp.	Rhizopus Spp.	Botrytis Cinerea
Provax 200 WP	14.75 f	6.25 bc	14.55 c	0.50 c	2.50 c	6.25 c	15.00 cd	1.25 e
Bavistin 70 WP	20.75 f	1.75 e	21.50 bc	1.00 c	4.50 c	2.50 c	23.75 bcd	0.75 e
Neem Leaf Extract	64.25 b	8.75 b	26.00 b	0.75 c	2.50 c	24.00 b	12.00 d	2.50 cd
Garlic Clove Extract	48.75 c	3.25 cde	24.00 b	3.35 bc	2.25 c	25.25 b	17.50 cd	3.25 c
Healthy Seeds	61.25 b	5.25 cd	24.00 b	2.5 c	2.00 c	29.75 b	25.75 bc	1.50 de
Bio-Fertilizer	30.25 e	2.25 de	15.25 c	0.75 c	2.00 c	31.50 b	31.50 b	3.25 c
Breeder Seed	36.50 d	2.25 de	23.00 bc	6.50 b	10.50 b	38.75 b	8.50 e	7.00 b
Control	81.00 a	12.25 a	42.25 a	10.25 a	16.50 a	63.00 a	45.25 a	11.25 a
LSD (0.05)	6.062	3.021	8.199	3.23	5.59	17.14	13	1.087
CV%	**6.86**	**9.33**	**12.46**	**7.51**	**8.9**	**12.59**	**13.18**	**14.71**

** Figures (s) in the column having the common letter(s) do not differ significantly at 5% levels.*

b) Radial growth inhibition of Sclerotium rolfsii

The result showed that Provax 200 WP at 200 and 400 ppm completely inhibited the radial growth of *Sclerotium rolfsii.* The % inhibition was significantly higher in all other treatment except garlic extract at 20% concentration. Garlic clove extract at 20% level also showed the effective result (96.26%) against the growth followed by Provax 200 WP at 100 ppm (94.44%). Bavistin 70 WP showed a negative outcome on Growth inhibition of *Sclerotium rolfsii* with the increase in concentration. The result showed that Bavistin 70 WP was less potent at high concentration rather than low level in reducing the radial growth of *Sclerotium rolfsii.* It inhibited the radial growth 27.04, 7.83 and 0.00% at 100, 200 and 400 ppm, respectively. In case of Neem extract at 5% level did not affect the radial growth inhibition of *Sclerotium rolfsii* and reduced only 5.56 and 32.22% at 10 and 20% level, respectively. Garlic clove extract inhibited the radial growth of *Sclerotium rolfsii* up to 6.72, 28.89 and 96.26% at 5, 10 and 20% level, respectively (Table 2).

Table 2: Effect of Treatments on the Percentage of Radial Growth Inhibition of *Sclerotium Rolfsii* on Farmers' Stored Chickpea Seeds

Treatments	Concentration (Dose)	% Inhibition
Provax 200 WP (ppm)	100	94.44 b
	200	100 a
	400	100 a
Bavistin 70 WP (ppm)	100	27.04 c
	200	7.83 d
	400	0.00 e
Neem Extract (% w/v)	5	0.00 e
	10	5.56 d
	20	32.22 c
Garlic Clove Extract (% w/v)	5	6.72 d
	10	28.89 c
	20	96.26 b
Control (Growth)	90 mm (0.00% inhibition)	

** Figure (s) in the column having a common letter(s) do not differ significantly at 5% level.*

c) Radial growth inhibition of Fusarium oxysporum

The results of the effect of selected PDA amended fungicides on the radial growth of *Fusarium oxysporum* are presented in Table 3. The result of the experiment showed that Bavistin 70 WP at all concentration significantly inhibited the radial progress of *Fusarium oxysporum* compared to all other treatments. Garlic clove extract at 5% level exhibited the lowest inhibition (20.41%). Provax at 400 ppm showed a better effectiveness (73.71%) against the *Fusarium oxysporum* to inhibit its growth but lower than those of Bavistin. It also results in 58.55 and 68.63% inhibition of growth at 100 And 200 ppm concentrations. Neem extract displayed in 35.72, 39.86 and 41.32% inhibition of growth at 5, 10 and 20% level respectively. Its performance however is less than those of Bavistin and Provax. Garlic clove extract inhibited the radial growth of *Fusarium oxysporum* having 20.41, 39.81 and 64.15% at 5, 10 and 20% level respectively.

Table 3: Effect of Treatments on the Percentage of Radial Growth Inhibition of *Fusarium Oxysporum* on Farmers' Stored Chickpea Seeds

Treatments	Concentration (Dose)	% Inhibition
Provax 200 WP (ppm)	100	58.55 d
	200	68.63 c
	400	73.71 b
Bavistin 70 WP (ppm)	100	100 a
	200	100 a
	400	100 a
Neem Extract (% w/v)	5	35.72 f
	10	39.86 ef
	20	41.32 e
Garlic Clove Extract (% w/v)	5	20.41 g
	10	39.81 ef
	20	64.15 c
Control (Growth)	86.00 mm (0.00% inhibition)	

** Figure (s) in the column having the common letter(s) do not differ significantly at 5% level.*

d) Radial growth inhibition of Botrytis cinerea

In case of *Botrytis cinerea*, all treatment showed the significant effect over control. The result of the laboratory experiment showed that both Provax 200 WP and Bavistin 70 WP significantly inhibited the radial growth of *Botrytis cinerea* at all selected concentrations compared to plant extract. Garlic clove extract showed the lowest effectiveness in inhibiting the growth of *Botrytis cinerea*. Neem Extract inhibited the radial growth of *Botrytis cinerea* 29.63, 32.22 and 38.52% at 5, 10 and 20% concentration respectively. In case of Garlic clove

extract, the inhibition percentages were 12.22, 17.04 and 31.48 at the concentration of 5, 10 and 20% respectively (Table 4).

Table 4: Effect of Treatments on the Percentage of Radial Growth Inhibition of *Botrytis Cinerea* on Farmers' Stored Chickpea Seeds.

Treatments	Concentration (Dose)	% Inhibition
Provax 200 WP (ppm)	100	100 a
	200	100 a
	400	100 a
Bavistin 70 WP (ppm)	100	100 a
	200	100 a
	400	100 a
Neem Extract (% w/v)	5	29.63 c
	10	32.22 c
	20	38.52 b
Garlic Clove Extract (% w/v)	5	12.22 e
	10	17.04 d
	20	31.48 c
Control (Growth)	90 mm (0.00% inhibition)	

** Figure (s) in the column having a common letter(s) do not differ significantly at 5% level*

e) Effect on plant population per plot

The plant population of chickpea under different seed treatments was recorded at 10, 20 and 60 days after sowing and the results are presented in Table 5. At 10 DAS the maximum plant population (336.8) per plot was observed in Provax 200WP treated seeds and Breeder seeds (333.5) while the minimum plant population (272.5) was found in the untreated plot. Garlic extract and healthy seeds + Bavistin 70WP showed a statistically dissimilar population per plot. Bavistin 70WP alone, Neem leaf extract and Bio-fertilizer + Bavistin 70WP showed statistically similar result. The highest plant population per plot at 20 DAS (320.0) and 60 DAS (306.0) was reported in Provax 200 WP treated plot while the lowest plant population at 20 DAS (224.0) and 60 DAS (183.0) was recorded in control.

Table 5: Effect of Treatment on Plant Population Grew from Farmers Stored Chickpea Seeds in the Field

Treatment	Plant Population / Plot		
	10 DAS	20 DAS	60 DAS
Provax-200 WP	336.8 a	320.0 a	306.0 a
Bavistin 70 WP	325.2 b	299.3 c	275.3 bc
Neem Leaf Extract	321.2 b	291.7 d	265.3 cd
Garlic Clove Extract	300.5 d	265.7 f	232.7 e
Healthy Seeds + Bavistin 70 WP	311.2 c	278.7 e	248.3 de
Bio-Fertilizer + Bavistin 70 WP	325.2 b	296.7 c	255.7 cd
Breeder Seed + Bavistin 70 WP	333.5 a	311.3 b	293.3 ab
Control	272.5 e	224.0 g	183.0 f
LSD (0.05)	7.153	4.99	19
CV (%)	**1.41**	**1.07**	**4.52**

** Figures (s) in the column having a common letter(s) do not differ significantly at 5% levels*

f) Foot and root rot control in both pot and field conditions

In Pot % infection was determined after 7, 14 and 21 DAS. At all DAS treatments showed significantly lower % incidence in the pot compared to that of control. After 7 DAS no infection was found in Provax 200 WP. At 14 DAS and 21 DAS, the Provax 200 WP treatment showed the lowest infection of 1.75% and 3.0%, respectively followed by Bavistin 70 WP and Healthy seeds which were statistically identical to those of Provax 200WP treated plot.

In the field, data were gathered at ten days' interval at 10DAS to 30 DAS. At 10 DAS the percent infection per m^2 was different for different treatments (Table 6). In case of control, the highest infection (27.88%) was recorded and the lowest infection (9.25%) per m^2 was found in the plots treated with Provax 200WP alone followed by Breeder seeds + Bavistin 70WP (12.14%), only Bavistin 70WP (12.88%), healthy seed + Bavistin 70WP (14.08%), Garlic extract (16.15%), Neem leaf extract (18.75%), Bio-fertilizer + Bavistin 70WP (19.43%) (Table 6).

Table 6: Performance of Different Treatments against Foot and Root of Chickpea in the Field

Treatment	% Infection in Pot			% Infection/m^2 in Field		
	7 DAS	14 DAS	21 DAS	10 DAS	20 DAS	30 DAS
Provax 200 WP	0.00 c	1.75 f	3.0 e	9.25 e	7.67 f	9.55 e
Bavistin 70 WP	1.5 b	2.5 ef	3.0 e	12.88 de	9.85 ef	11.27 de
Neem Leaf Extract	2.0 b	4.0 cde	7.0 cd	18.75 bc	11.52 de	14.63 bc
Garlic Clove Extract	1.0 bc	4.25 bcd	9.0 bc	16.15 bcd	12.80 cde	12.86 bcd
Healthy Seeds + Bavistin 70 WP	2.0 b	3.0 def	5.0 de	14.08 cde	15.49 bc	11.91 cde
Bio-Fertilizer + Bavistin 70 WP	1.0 bc	5.75 b	11.0 b	19.43 b	18.81 b	15.69 b
Breeder seed + Bavistin 70 WP	2.0 b	5.0 bc	8.0 bcd	12.14 de	14.09 cd	11.88 cde
Control	3.5 a	7.75 a	15.0 a	27.88 a	28.45 a	19.21 a
LSD (0.05)	1.16	1.52	3.76	4.802	3.424	3.22
CV%	**11.23**	**6.83**	**8.57**	**16.8**	**13.18**	**14.34**

** Figures (s) in the column having the common letter(s) do not differ significantly at 5% levels.*

g) Performance of different treatments against Fusarium wilt

The incidence of Fusarium wilt at three dates after sowing following treatments with divergent chemicals, botanicals using healthy seeds and breeder seeds has shown in Table 7. At 45 DAS, the mean number of Fusarium-infected plant per plot (11.33 plants) was the highest in untreated control plot and the lowest in Bavistin 70 WP (4.33 plants) treated plot followed by Provax 200 WP (4.67 plant), apparently healthy seeds (5.33 plants) and breeder seeds (5.33 plants). At 60 DAS, again the highest number (12.67) of *Fusarium* infection occurred in control plot and the lowest in Bavistin 70 WP (5.00) followed by Provax 200 WP (5.67 plants), using healthy seeds (6.67 plants) and breeder seeds (7.00 plants). At 75 DAS, Bavistin 70 WP exhibited the lowest (3.33 plants) infected plant per plot followed by Provax 200 WP (4.00 plants), apparently healthy seeds (5.00 plants) and breeder seeds (5.33 plants). The highest *Fusarium* infection was noted in control plot (12.67 plants).

Table 7: Effect of Different Treatments on Fusarium Wilt in Field Condition

Treatment	Plant Population / Plot		
	45 DAS	60 DAS	75 DAS
Provax-200 WP	4.67 d	5.67 d	4.00 ef
Bavistin 70 WP	4.33 d	5.00 d	3.33 f
Neem Leaf Extract	6.01 bcd	8.00 bc	6.67 bcd
Garlic Clove Extract	7.33 b	8.33 bc	7.00 bc
Healthy Seeds + Bavistin 70 WP	5.33 cd	6.67 cd	5.00 def
Bio-Fertilizer + Bavistin 70 WP	6.67 bc	9.00 b	7.67 b
Breeder Seed + Bavistin 70 WP	5.33 cd	7.00 bcd	5.33 cde
Control	11.33 a	12.67 a	12.67 a
LSD (0.05)	1.682	1.934	1.703
CV (%)	**15.07**	**14.18**	**15.06**

* Figures (s) in the column having a common letter(s) do not differ significantly at 5% levels.

h) Grey mold control in field condition

All the treatment showed the significantly dissimilar outcomes over untreated plot. At 65 DAS significantly the highest number (14 plants) was observed in untreated control plot and the lowest in Bavistin 70 WP (2.67 plants) treated plot followed by Provax 200 WP (3.00 plants) but they were statistically similar. Utilization of apparently healthy seeds and breeder seeds had *Botrytis* gray mold infection of 5.33 plants/plot and 6.67 plants/plot at 65 DAS. At 80 DAS, significantly the highest number (16.33 plants) of infected plant found in control plot and the lowest in Provax 200 WP (5.33 plants) followed by Bavistin 70 WP (5.67 plants). At 95 DAS, the lowest number (3.33 plants) of infected plant found in Provax 200 WP followed by Bavistin 70 WP (4.67 plants) which are statistically alike. Healthy seed + Bavstin 70WP treated plot exhibited 5.00 plants/plot infections by BGM which was statistically similar to Bavistin 70WP (4.67). Garlic extract and neem extract resulted in 8.67 and 7.33 BGM infected plants per plot, respectively at 95 DAS which were statistically similar (Table 8).

Table 8: Performance of Different Treatments against Botrytis Gray Mold of Chickpea

Treatment	Plant Population / Plot		
	65 DAS	80 DAS	95 DAS
Provax-200 WP	3.00 f	5.33 e	3.33 f
Bavistin 70 WP	2.67 f	5.67 e	4.67 ef
Neem Leaf Extract	7.67 cd	9.00 cd	7.33 cd
Garlic Clove Extract	9.00 c	10.33 c	8.67 c
Healthy Seeds + Bavistin 70 WP	5.33 e	7.67 d	5.00 e
Bio-Fertilizer + Bavistin 70 WP	11.67 b	12.33 b	10.33 b
Breeder Seed + Bavistin 70 WP	6.67 de	7.67 d	6.67 d
Control	14.00 a	16.33 a	15.33 a
LSD (0.05)	1.351	1.62	1.57
CV (%)	**10.29**	**12.63**	**14.24**

** Figures (s) in the column having the common letter(s) do not differ significantly at 5% levels*

IV. Discussion

The experiments were conducted following seed treatment in blotter method, pot and field spray with fungicides, botanicals, bio-fertilizer and healthy seeds. Results of the study reveals that all treated seeds including healthy seeds showed significant performance against associated fungi compared to control. However, fungicides significantly inhibited growth of fungi in culture media, *Sclerotium rolfsii* in pot and field incidence of all pathogens. In blotter method, Provax 200WP had shown significant performance compared to other treatments. Untreated seeds showed the maximum occurrence of seed-borne fungi. Among the tested fungicides, Provax 200 WP appeared to be the best in inhibiting the radial growth of the pathogen *Sclerotium rolfsii* at 200 and 400 ppm concentration.

Garlic clove Extract at 20% was effective. Except Bavistin 70 WP at 400 ppm and Neem Extract at 5% level all other treatments showed effective result against the *Sclerotium rolfsii*. Bavistin 70 WP exhibited results and may need a special consideration before set the applying concentration against *Sclerotium rolfsii.* Bavistin 70 WP appeared to be the best in inhibiting the radial growth of the pathogen *Fusarium oxysporum* at all selected concentration. Provax 200 WP also showed the significant result but better at 400 ppm. In case of both Neem and Garlic clove extract, at 20% level showed the more effectiveness compared to 5 and 10% concentration. It seems that Provax 200 WP and Bavistin 70 WP were the best radial growth inhibiting fungicides at all concentration compared to Neem Extract and Garlic clove Extract in inhibiting the radial growth of *Botrytis cinerea* at all its concentration. It reveals that Vitavax-200 played appreciable role in controlling *Sclerotium rolfsii*, *Fusarium oxysporum*, *Botrytis cinerea* etc. Also, Bavistin 70WP successfully protected *Fusariumm oxysporum* in culture and field condition, and considerably reduced other fungal prevalence. In controlling field incidence of Foot and root rot at 20 DAS and 30 DAS Provax 200 WP showed significantly the highest performance but statistically similar to those of Bavistin 70WP respectively. The results showed that in all parameters Provax 200 WP performed the best compared to other treatments. Healthy seed + Bavistin 70WP showed moderate efficiency but significantly better than untreated control.

Many authors have reported that fungicides efficiently reduce infection and vitavax-200 was the most effective in controlling the seed-borne fungi (Tewari *et al*., 2003; Salam, 2004; Haque *et al*., 2009; Masum *et al*., 2008; Behrani *et al*., 2015; Saranya *et al*., 2017). Between the two botanical extracts, garlic clove extracts performed better than neem extracts. In case combinations of Bavistin 70WP with healthy seeds, bio-fertilizer and breeder seeds; the Bavistin 70WP and healthy seeds exhibited better performance. A good number of researchers showed that the plant extracts were a potential agent for the control of seed-borne pathogens and also improved germination of various seed (Hawlader, 2003; Sinha *et al*. 2004; Riazuddin *et al*., 2009). There are reports that garlic and neem extracts appreciably inhibited radial growth and spore germination of *Fusarium* spp. (Ahmed and Islam, 2000; Mondall *et al*., 2009; Perelló *et al*., 2013; Awad, 2014). Bio-fertilizers significantly control seed-borne diseases and able to enhance plant growth (Hossain *et al*., 1999; Khan *et al*., 1998; Rahman *et al*., 2006; Kibria and Hossain, 2004; Khalequzzaman, 2015). It reveals that fungal prevalence depicted an opposite relation with time and concentration as incidence increased with prolongation, but decreased with increasing concentration of different treatments. These finding agreed well with Khan *et al*., 2017 who has reported reduced incidence with increasing storage period after fungicidal treatment as toxicity of the chemicals declined.

V. Conclusions

It is clear that fungicides acted as a safeguard to control the prevalence of foot and root rot, Fusarium wilt, gray mold etc. diseases of farmers' stored chickpea. Provax 200WP and Bavistin 70WP may be considered as the best seed treating agent for health and quality maintenance during storage and in field conditions. Also, the use of Healthy seeds with Bavistin 70WP spray may be considered as an efficient practice. Combination of botanicals, bio-agents and healthy seeds with fungicides may be suggested as part of integrated control, but had less impact on fungal growth inhibition.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 4 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Prevalence, Risk Factors and Major Bacterial Causes of Bovine Mastitis in Smallholder Dairy Farms in and around Sinana District, Bale Zone, South Eastern Ethiopia

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Abstract- A cross-sectional study was conducted from November 2013 to May 2014 on lactating dairy cows to determine the overall prevalence of bovine mastitis, identify associated risk factors and isolate the predominant bacterial agents involved in causing mastitis in and around Sinana district. A total of 384 lactating cows were examined for mastitis using clinical examination and California Mastitis Test (CMT). Bacteriological isolation techniques were also undertaken to recover the causative bacterial pathogens. Prevalence of mastitis at cow level was 36.72%, out of which 4.95% and 31.77% were clinical and subclinical cases, respectively. The quarter level prevalence was 26.43%; from this, the clinical and subclinical forms were 2.28% and 24.15%, respectively. Out of total examined teats, 1.30% was blind. About 356 bacterial isolates identified from mastitic milk samples. The isolates based on their relative frequency of occurrence were: Staphylococcus aureus (33.99%), Streptococcus agalactiae (24.44%), Staphylococcus epidermidis (10.96%), Coagulase-Negative Staphylococci (CNS) (7.58%), Escherichia coli (6.46%), Streptococcus dysgalactiae (6.18%), Corynebacterium bovis (5.34%), Klebsiella pneumonia (2.81%) and Bacillus cereus (2.23%).

Keywords: *bacterial isolates, bovine mastitis, lactating cow, prevalence, risk factors, sinana.*

GJSFR-D Classification: *FOR Code: 070199*

Strictly as per the compliance and regulations of:

Prevalence, Risk Factors and Major Bacterial Causes of Bovine Mastitis in Smallholder Dairy Farms in and around Sinana District, Bale Zone, South Eastern Ethiopia

Kemal Kedir Elemo [α], Birihanu Abera Bedada [σ] & Taye Kebeda [ρ]

Abstract- A cross-sectional study was conducted from November 2013 to May 2014 on lactating dairy cows to determine the overall prevalence of bovine mastitis, identify associated risk factors and isolate the predominant bacterial agents involved in causing mastitis in and around Sinana district. A total of 384 lactating cows were examined for mastitis using clinical examination and California Mastitis Test (CMT). Bacteriological isolation techniques were also undertaken to recover the causative bacterial pathogens. Prevalence of mastitis at cow level was 36.72%, out of which 4.95% and 31.77% were clinical and subclinical cases, respectively. The quarter level prevalence was 26.43%; from this, the clinical and subclinical forms were 2.28% and 24.15%, respectively. Out of total examined teats, 1.30% was blind. About 356 bacterial isolates identified from mastitic milk samples. The isolates based on their relative frequency of occurrence were: *Staphylococcus aureus* (33.99%), *Streptococcus agalactiae* (24.44%), *Staphylococcus epidermidis* (10.96%), Coagulase-Negative Staphylococci (CNS) (7.58%), *Escherichia coli* (6.46%), *Streptococcus dysgalactiae* (6.18%), *Corynebacterium bovis* (5.34%), *Klebsiella pneumonia* (2.81%) and *Bacillus cereus* (2.23%). Risk factors analysis revealed that prevalence of mastitis was significantly differed with the age (P< 0.01), parity (P< 0.05), breed (p<0.001), stage of lactation (p<0.001), mastitis record (p<0.01), dry cow therapy (p<0.05), udder hygiene (p<0.01), drainage system (p<0.05), floor type (p<0.05) and grazing system (P<0.05). Thus, prevalence was relatively higher in adult cows (OR = 1.784; 95% CI = 0.999, 3.189), multiparous cows (OR = 1.320; 95% CI = 0.552, 3.155), cross breed cows (OR = 5.820, 95%CI = 3.248, 10.430), early stage lactation (OR=3.021, 95%CI=1.617, 5.647), late stage lactation (OR = 3.280, 95%CI = 1.931, 5.572), cows with history of mastitis (OR = 2.452, 95%CI = 1.282, 4.688), cows untreated during drying off (OR=1.445, 95%CI=0.467, 4.473), cows with unwashed udder (OR = 13.386, 95% CI = 1.300, 137.845) and cows under zero grazing (OR=1.892, 95%CI=1.022, 3.501) than those corresponding animals. Generally, the study showed that mastitis is an important problem and a serious threat for the dairy industry in the study area. Therefore, appropriate control measures targeting the specific causative agents should be in place to reduce the impact of the disease. The farmers should have to implement sound management practices that improve udder and teat heath problems.

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Keywords: bacterial isolates, bovine mastitis, lactating cow, prevalence, risk factors, sinana.

I. Introduction

Bovine mastitis is the inflammation of the mammary gland often due to microorganisms that attack the udder, proliferate and release toxins that are injurious to the udder and teat tissues (Schroeder, 2012). It has been a disease of cattle for probably as long as humankind has milked cows (Erskine *et al*., 2002). Mastitis is among the most significant diseases in dairy animals with worldwide distribution (Zhao and Lacasse, 2007). It is manifested by un array of physical and chemical alterations in the milk and pathological lesions in the glandular tissue (Radostits *et al*., 2007). It is a global problem responsible for massive financial losses to dairy industries and economies at large due to poor milk quality, reduced milk yield and increased expenditure on treatment and sometimes death due to the disease itself or through culling of affected cows (Schroeder, 2012).

Numerous microorganisms have been described as causative agents of bovine mastitis (Watts, 1988; Bradley, 2002). According to their epidemiology, mastitis pathogens can be divided into contagious and environmental. The primary reservoir of contagious pathogens is an infected udder whereas a contaminated environment is the primary reservoir of pathogens causing environmental mastitis. *Staphylococcus aureus, Streptococcus agalactiae*, and Mycoplasma species are considered as typical contagious pathogens. Typical environmental pathogens are streptococci (streptococci other than *Streptococcus agalactiae* such as *Streptococcus uberis*; enterococci), Enterobacteriaceae and coagulase-negative staphylococci (CNS). *Streptococcus dysgalactiae* has been most commonly considered as a contagious pathogen, but it can also act as an environmental pathogen (Gruet *et al*., 2001; Bradley 2002; Barkema *et al*., 2009). Likewise, the contagious infection has also been recorded in certain coagulase-negative staphylococci (CNS) (Gillespie *et al*., 2009). Pathogens such as Pseudomonas species, Pasteurellaceae, some pyogenic and anaerobic

bacteria, yeasts and algae number among those which occur occasionally. In current times, there is obvious confirmation for rising occurrence of environmental mastitis while the incidence of contagious mastitis has decreased (Bradley 2002; Rysanek *et al*., 2007).

Intra-mammary infections (IMI) can result in mastitis which is either sub-clinical or clinical. Clinical mastitis is type of mammary tissue infection that can be directly seen, with signs such as alterations in milk composition and appearance; reduction in milk production; affected udder/teats become red, hard, hot and swollen. In addition, it is manifested by symptoms like increased in body temperature, rapid pulse, loss of appetite, depression and sometimes death. Sub-clinical mastitis is generally defined as the absence of visible symptoms but characterized by cell count (SCC) of greater than 2.5 x105 cells/ml (Schukken *et al.,* 2003) or the presence of a known pathogen in the secreted milk as detected by culture. Subclinical form commonly found in most herds (Gruet *et al.,* 2001; Awale *et al*., 2012). Clinical mastitis is mainly caused by pathogens such as *Streptococcus uberis*, *Escherichia coli*, Klebsiella species, *Pseudomonas aeruginosa* and pyogenic bacteria. On the other hand, *Streptococcus agalactiae*, Coagulase-negative Staphylococci (CNS) and Enterococcus species are associated with subclinical mastitis (Bradley 2002; Barkema *et al*., 2009; Awale *et al*., 2012). However, *Staphylococcus aureus* has been considered as the cause of both clinical (Gruet *et al*., 2001) and subclinical mastitis (Awale *et al*., 2012). In contrary to the clinical form of the disease, subclinical mastitis is difficult to recognize, and for this reason, it may result in heavy losses in milk yield. In addition, subclinically affected cows might represent a source of particular pathogens that can be spread via automatic milking systems (Barkema *et al*., 2009; Hovinen and Pyorala 2011).

The incidence of mastitis is significantly influenced by environment and management related factors (Steeneveld *et al*., 2008; Ali *et al*., 2014). The occurrence of mastitis depends on three components which include exposure to microbes, cow defense mechanism, environmental and management factors (Suriyathaporn *et al*., 2000). The early months of lactation is the most sensitive period for mastitis risk in the cow even in the well-managed herds (Andrew *et al*., 2004). Numerous risk factors with bovine mastitis are associated microflora of the udder, udder shape and condition, teat injuries, teat length, increasing teat canal diameter, udder depth, teat morphology (Tiwari *et al*., 2013; Ali *et al*., 2014). Majority of diagnosed mastitis cases are the result of bacterial infections. A major survey of New York and Pennsylvania dairy herds found that almost 50% of all cows were experiencing some form of mastitis caused by a culturable microorganism; less than 1 % of these were due to a non-bacterial pathogen (Wilson *et al.,* 1997). These pathogens invade the mammary glands, develop and multiply, producing some toxic substances that result in inflammation, reduced milk production and altered milk quality leading to a clinical condition known as mastitis (Oliver and Muranda, 2012; Rall *et al*., 2013).

The existing literatures revealed that udder and teat disease is one of the most regularly encountered diseases of dairy cattle. Investigation conducted by Lemma *et al*. (2001) showed, of the main diseases of cross breed cows in Addis Ababa milk shed, clinical mastitis was the second most frequent next to reproductive disease. Mastitis, as a disease, has received little attention in Ethiopia, especially the sub clinical form (Mekonnen *et al*., 2005; Hundera *et al*., 2005) which occurs at a much higher rate than clinical mastitis, yet it is the nastiest in terms of reduced productivity (Quinn *et al*., 2002). Owing to the serious financial insinuation involved and the predictable existence of latent infection, mastitis is the vital factor that limits dairy industry. There are various reports indicating a high prevalence of bovine mastitis in dairy farms in different parts of Ethiopia (Mekibib *et al*., 2010; Bedada and Hiko, 2011; Fentaye *et al*., 2014; Tilahun & Aylate, 2015; Teklemariam *et al*., 2016).

Although various investigations have been conducted on bovine mastitis in Ethiopia so far, the problem is still challengeable for the bovine mastitis researchers and particularly for field veterinarians to treat and control it. Now there is a need to imply the strategic control measures for this deadly disease of dairy animals to prevent heavy economic losses of farmers. We need distribution and changing trend of etiological agents, prevalence and potential risk factors of mastitis in the study area to apply strategic plan for control of mastitis. Moreover, there is no published data on status, magnitude, and distribution of mastitis in Bale Zone in general and in and around Sinana district in particular. Hence, the aim of this investigation is to establish the distribution of etiological agents, prevalence and potential risk factors of bovine mastitis from the study area.

II. Materials and Methods

a) *Description of the Study Area*

The study was conducted in and around Sinana district of Bale zone, Oromia Regional State, South Eastern Ethiopia. It is located at 430 km south-east of Addis Ababa. The area is located at 7O7' N and 40O10' E and 2400 meters above sea level. The mean average rainfall of the district is 353 mm. Moreover, an average annual maximum temperature is 21.2oC, and the minimum temperature is 9.4oC. The agricultural production system of the study area is mixed farming. There are about 251,489 heads of cattle, of which 59,561 are dairy cows, 47,121 Sheep, 10,300 goats, 9,163 horses, 14,015donkey, 2,800 mules, 59,655

poultry and 13,690 beehives in Sinana woreda (Sinana Woreda Agricultural and Rural Development Office, 2013). Dairy farming using local and improved (cross) breeds is a common practice in Sinana district where dairy production plays a crucial role in the livelihood of the farming community. The management system of dairy cows is mainly extensive in rural areas and intensive in town. Traditional housing, feeding and milking procedures are mostly practiced.

b) Study Population and Animals

The study populations were all lactating cows from Sinana district. The breeds of animals were the local zebu (predominant) and the zebu crossbred with Holstein-Friesian. The study animals consisted of 384 milking cows, 308 indigenous zebu, and 76 Holstein-zebu crosses, selected by simple random sampling method from smallholder dairy farms in chosen kebeles. All the study cows were hand milked and milked twice a day.

c) Study Design

A cross-sectional type of study supported by laboratory tests was carried out to determine the prevalence, major bacterial causes and to assess risk factors of bovine mastitis at the cow and quarter level from October 2013 to May 2014 on small holder dairy farms in and around Sinana district. Cows were examined directly at the quarter level for clinical manifestations and indirect tests (CMT) for subclinical mastitis.

d) Sampling Method and Determination of Sample Size

Sampling was accomplished using the simple random sampling technique to choose individual dairy cow. The sample size required for the study was calculated according to the formula given by Thrusfield (2007) for simple random sampling.

$$n = \frac{(1.96)^2 P_{exp} (1 - P_{exp})}{d^2}$$

Where: n = required sample size,
Pexp = expected prevalence, and
d = desired absolute precision

Due to absence of logical research work undertaken in this district so far; the sample size is calculated using a technique suggested by Thrusfield (2007), with 95% confidence interval, at 5% desired absolute precision and expected prevalence of 50%. Hence; the total numbers of sample needed for this observation was 384 lactating dairy cows. Since the prevalence of mastitis was not known previously in the area, six kebeles (lowest administrative structure) were randomly selected using a lottery system out of the ten kebeles with a high number of dairy cows in the district. Proportionality of incorporating cattle in the sample will be applied as per the population size of each district and kebeles.

Table 1: Proportional allocation and number of animals sampled from each kebeles.

Kebeles	Number of Lactating Cows in the Kebeles	No. of Lactating Cows Sampled (Calculated Sample Size)
Basaso	2186	72
Nanno Robe	2058	66
Shallo	1855	61
Hora Boka	2102	69
Kabira Shaya	2339	77
Donsa	1150	39
Total	**11690**	**384**

Source: Data obtained from Sinana "Woreda" Agricultural Office (2013).

e) Sample collection and bacteriological examination

i. *Collection of milk samples*

Milk samples were collected according to the standard procedures recommended by National Mastitis Council NMC (2004). Approximately 10 ml of milk was collected aseptically from lactating cows into sterile test tubes after discarding the first three milking streams. Samples from each quarter were transported in the ice box (4°C) to Microbiology Laboratory of Debra Zeit School of Veterinary Medicine and Agriculture, where they were immediately cultured or stored at 4°C until processed or cultured on standard bacteriological media.

f) Examination of Clinical Mastitis

Clinical cases were recorded at the time of milk sampling. Clinical mastitis was diagnosed by the manifestation of visible signs of inflammation and abnormal milk. A quarter, which is warm, swollen and painful for the cow upon palpation was considered to have acute clinical mastitis; whereas atrophied, hard and fibrotic quarters were considered to have chronic mastitis (Quinn *et al.,*2004; Radostitits *et al.*, 2007).

g) California Mastitis Test screening

California Mastitis Test was performed for each quarter of a lactating cow. It is used to determine the prevalence of sub-clinical mastitis and also as the screening test for selection of samples to be cultured for the cows under study. A small sample of milk (approximately ½ teaspoon) from each quarter was collected into a plastic paddle that has four shallow cups marked A, B, C and D. An equal amount of California Mastitis Test reagent was added to the milk. The paddle was rotated to mix the contents. The CMT result was interpreted as negative (0), trace (T), weakly positive (+1), distinct positive (+2) and strongly positive (+3) as per the recommendation which is given by Quinn *et al*. (2004). Cows were considered positive for CMT when at least one quarter turned out to be positive for CMT. A herd was considered positive for CMT when at least one cow in a herd is tested positive for CMT.

h) Bacteriological examination of milk samples

i. *Cultural procedures and biochemical tests*

Isolation and identification of mastitis pathogens were conducted in the Microbiology Laboratory of Bishoftu, College of Veterinary Medicine and Agriculture. The bacteriological culture was executed following the standard microbiological techniques recommended by Quinn *et al.* (2004), National mastitis council (NMC) (2004). A loop full of milk was streaked on 5% sheep blood agar, nutrient agar, and MacConkey agar and then, the plates were incubated aerobically at 37 OC and examined after 24hrs of incubation for growth. The colonies were provisionally identified by staining reaction with Gram's stain, cellular morphology, colony morphology, pigmentation and hemolytic pattern on blood agar and other environment from which the bacterium was isolated. Subcultures were done to obtain pure isolates for further identification. In doing so, the representative colonies were subcultured on blood agar plate and nutrient slants and incubated at 37 OC. The slants were preserved and maintained for characterizing the isolates. Identification was done according to the standard methods described by Quinn *et al*. (2004).

i) Questionnaire survey of risk factors

Data was collected using a semi-structured questionnaire. The questionnaire was prepared, pre-tested and adjusted by translating into local language and administered by the same interviewer (researcher) who speaks the same language with the participant smallholders with the primary objective of elucidating the multifactorial background of mastitis. Data collected include intrinsic factors such as age, breed, parity, stage of lactation, previous history of mastitis and body condition. Extrinsic factors such as dry cow therapy, udder hygiene, drainage system, floor type and grazing system were also recorded.

j) Data Storage and Analysis

All data from laboratory tests and questionnaire were entered into a Microsoft Excel spreadsheet and accuracy was checked for statistical evaluation. After validation, data were transferred to STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA) for analysis. The dependent variable suggested in the data analysis was mastitis status of a cow and the potential risk factors considered were parity of the cow, stage of lactation, breed, age, previous mastitis history and floor type. Prevalence was estimated as a percentage value. The relationship between the potential risk factors and the prevalence of mastitis was evaluated using the Chi-square test ($\chi 2$). Multivariate logistic regression analyses were used to analyze the effects of different supposed risk factors on the prevalence of mastitis. Odds ratio (OR) was utilized to determine the degree of association between putative risk factors with mastitis prevalence. The 95% confidence interval and a p-value <0.05 was considered statistically significant.

III. Results

a) Prevalence of mastitis

A total of 384 lactating cows (308 local and 76 crossbreed) were examined for mastitis detection. Out of the total examined, prevalence of mastitis at cow level was 36.72% (141/384), out of which 4.95% (19/384) and 31.77% (122/384) were clinical and sub clinical, respectively. A total of 1536 quarters were considered in this study and the quarter level prevalence was 26.43% (406/1536), from which 2.28% (35/1536) and 24.15% (371/1536) were found to be of clinical and subclinical forms, respectively (Table 2). Out of the 35 quarters with clinical cases, 1.30% (20/1536) was blind teats. The remaining, 0.98% (15/1536), was of a clinical form showing active cases of mastitis with manifested symptoms of inflammation on the udder and teat; and alterations in milk quality.

Table 2: Prevalence of mastitis at the cow and quarter level.

Forms of Mastitis	Total Numbers Examined	Total Numbers Affected (%)
Clinical		
Cow Level	384	19 (4.95)
Quarter Level	1536	35 (2.28)
Subclinical		
Cow Level	384	122 (31.77)
Quarter Level	1536	371 (24.15)
Overall		
Cow Level	384	141 (36.72)
Quarter Level	1536	406 (26.43)

In quarter level prevalence of subclinical mastitis, right rear teats (RR) showed the highest rate of infection (27.15%) followed by the left rear quarters (LR), 25.67%; left front teats (LF), 23.61% and the right front quarters (RF), 22.49% (Table 3).

Table 3: Quarter level prevalence of subclinical mastitis (Functional teats = 1501).

Quarter	No. Examined	Positive	Frequency (%)
RF	378	85	22.49
RR	372	101	27.15
LF	377	89	23.61
LR	374	96	25.67
Total	**1501**	**371**	**24.72**

RR, right rear; RF, right front; LR, left rear and LF, left front.

The number of lactating cows examined within each six study kebeles and percentages found to be positive for mastitis is depicted in Table 4. Mastitis prevalence in selected kebeles was highest in Donsa followed by Basaso, Nanno Robe, Hora Boka, Shallo and Kabira Shaya. There were no significant differences between the chosen kebeles of the investigated district and mastitis prevalence.

Table 4: Prevalence of bovine mastitis within the selected kebeles.

Sampled Kebeles	Number of Lactating Cows Examined	Number of Positive Cows	Prevalence (%)
Basaso	72	31	43.06
Nanno Robe	66	27	40.91
Shallo	61	16	26.23
Hora Boka	69	25	36.23
Kabira Shaya	77	19	24.68
Donsa	39	23	58.97
Total	**384**	**141**	**36.72**

b) Intrinsic risk factors associated with the prevalence of bovine mastitis

A Chi-square analysis revealed that prevalence of bovine mastitis was significantly associated with the age groups ($P<0.004$), parity ($P<0.05$), breed ($P<0.001$), stage of lactation ($P<0.001$), mastitis record ($P<0.001$) and udder hygiene ($P<0.01$). However, its association with body condition was not significantly varied ($P>0.05$) (Table 5).

Table 5: Chi-square analysis of intrinsic risk factors associated with the occurrence of mastitis.

Factor	Category	No. Examined	No. Positive	Prevalence (%)	$\chi 2$ (P Value)
Age	≤ 5 Years	134	36	26.87	
	> 5 Years	250	105	42.0	8.600 (0.003)
Parity	Primiparous	52	12	23.08	
	Multiparous	332	129	38.86	4.817 (0.028)
Breed	Local	308	89	28.89	
	Cross	76	52	68.42	40.984 (0.000)
Stage Of Lactation	Early (< 3 Months)	68	32	47.06	
	Mid (3–5 Months)	196	48	24.49	
	Late (> 5 Months)	120	61	50.83	26.032 (0.000)
Mastitis Record	No	331	112	33.84	
	Yes	53	29	54.72	8.572 (0.003)
Body Condition	Poor	146	56	38.36	0.970 (0.616)
	Medium	137	52	37.96	
	Good	101	33	32.67	

The results of logistic regression analysis of the association of different risk factors with the prevalence of bovine mastitis are depicted in Table 6. Analysis of the association of intrinsic risk factors with the prevalence using multivariable logistic regression showed that cross-breeds (OR=5.820, 95%CI: 3.248,10.430), early-stage lactation (OR = 3.021, 95%CI: 1.617, 5.647), late-stage lactating cows (OR=3.280, 95%CI: 1.931, 5.572) and previous mastitis record (OR=2.452, 95%CI: 1.282,4.688) were at higher risk of infection with bovine mastitis as compared to local breed, mid-stage lactation and non previous mastitis record, respectively.

Table 6: Multiple logistic regression analysis to predict the intrinsic risk factors associated with mastitis.

		Mastitis Test Tesult		Odds Ratio		
Factor	Category	No. Examined	No. Positive (%)	COR (95% CI)	AOR (95% CI)	P Value
Age	≤ 5 Years	134	36 (26.87)	1	1	
	> 5 Years	250	105 (42.0)	1.971 (1.248, 3.114)	1.784 (0.999, 3.189)	0.051
Parity	Primiparous	52	12 (23.08)	1	1	
	Multiparous	332	129 (38.86)	2.118 (1.071, 4.189)	1.320 (0.552, 3.155)	0.532
Breed	Local	308	89 (28.89)	1	1	
	Cross	76	52 (68.42)	5.331 (3.098, 9.175)	5.820 (3.248, 10.430)	0.000
Stage Of Lactation	Mid (3–5 Months)	196	48 (24.49)	1	1	
	Early (< 3 Months)	68	32 (47.06)	2.741 (1.539, 4.880)	3.021 (1.617, 5.647)	0.000
	Late (> 5 Months)	120	61 (50.83)	3.188 (1.965, 5.171)	3.280 (1.931, 5.572)	0.000
Mastitis Record	No	331	112 (33.84)	1	1	
	Yes	53	29 (54.72)	2.363 (1.314, 4.249)	2.452 (1.282, 4.688)	0.007

COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; 1, Reference

c) *Extrinsic Risk Factors associated with the prevalence of bovine mastitis*

Management factors such as hygiene, dry cow therapy, housing, and grazing system were evaluated as extrinsic risk factors that influence the prevalence of bovine mastitis. The association between the occurrence of mastitis and extrinsic risk factors is presented in Table 7. Accordingly, mastitis prevalence showed significant variation with dry cow therapy (p = 0.021), udder/ teat hygiene (p = 0.001), drainage system (p= 0.033), floor type (p= 0.010) and grazing system (p=0.026).

Table 7: Chi-square analysis of extrinsic risk factors associated with the occurrence of mastitis.

Factor	Category	No. Examined	No. Positive	Prevalence (%)	$\chi 2$ (P Value)
Dry Cow Therapy	No	351	135	38.46	5.339(0.021)
	Yes	33	6	18.18	
Udder / Teat Hygiene	Poor	319	129	40.44	11.224(0.001)
	Good	65	12	18.46	
Drainage System	Poor	324	125	38.58	4.539(0.033)
	Good	64	16	25.00	
Floor Type	Soil	318	126	39.62	6.714(0.010)
	Concrete	66	15	22.73	
Grazing System	Zero Grazing	49	25	51.02	4.944(0.026)
	Grazing	335	116	34.63	

Risk factors logistic regression analyses showed that poor udder/teat hygiene had a significant effect (P<0.05) on the prevalence of mastitis. Bovine mastitis was more likely to occur in cows with poor udder/teat hygiene (OR = 13.386, 95%CI = 1.300, 137.845). Similarly, cows managed under zero grazing were more liable to mastitis (OR = 1.892, 95%CI = 1.022, 3.501) than cows under grazing. Odds of cows not receiving therapy during drying off was 1.445 times than those with dry cow therapy (Table 8).

Table 8: Multivariable logistic regression analysis of extrinsic risk factors associated with bovine mastitis.

		Mastitis Test Result	Odds Ratio		
Variable	Category	No. Positive (%)	COR (95% CI)	AOR (95% CI)	P Value
Dry Cow Therapy	No	135 (38.46)	2.812 (1.132, 6.990)	1.445 (0.467, 4.473)	0.523
	Yes	6 (18.18)	1	1	
Udder / Teat Hygiene	Poor	129 (40.44)	2.999 (1.542, 5.833)	13.386 (1.300, 137.845)	0.029
	Good	12 (18.46)	1	1	
Drainage System	Poor	125 (38.58)	1.923 (1.046, 3.535)	0.830 (0.323, 2.134)	0.698
Floor Type	Soil	126 (39.62)	2.231 (1.203, 4.139)	0.203 (0.022, 1.881)	0.161
	Concrete	15 (22.73)	1	1	
Grazing System	Zero Grazing	25 (51.02)	1.967 (1.075, 3.596)	1.892 (1.022, 3.501)	0.042
	Grazing	116 (34.63)	1	1	

COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; 1, Reference.

d) *Bacterial Isolates*

From 343 positive culture samples, a total of 364 bacterial isolates were recovered. The most prevalent culture growth was *Staphylococcus aureus* (33.24%) followed by *Streptococcus agalactiae* (22.25%), *Staphylococcus epidermidis* (9.34%), *E.coli* (7.42%), Coagulase-Negative Staphylococci (CNS) (7.14%), *Streptococcus dysgalactiae* (5.77%),

Corynebacterium bovis (4.40%), *Streptococcus uberis* (3.85%), *Klebsiella pneumonia* (2.75%), *Pseudomonas aeruginosa* (2.2%) and *Bacillus cereus* (1.65%) (Table 9).

Table 9: Frequency and proportion of bacterial species isolated from bovine mastitis (number of isolates= 356).

Bacterial Species	Total Number of Isolates	Prevalence (%)
Staphylococcus Aureus	121	33.24
Streptococcus Agalactiae	81	22.25
Staphylococcus Epidermids	34	9.34
Escherichia Coli	27	7.42
Coagulase Negative Staphylococci	26	7.14
Streptococcus Dysgalactiae	21	5.77
Corynebacterium Bovis	16	4.40
Streptococcus Uberis	14	3.85
Klebsella Pneumonae	10	2.75
Pseudomonas Aeruginosa	8	2.20
Bacillus Cereus	6	1.65
Total	364	100.00

IV. Discussion

The present study revealed that the overall prevalence of bovine mastitis at cow level was 36.72%. This is comparable with the previous findings of Workineh *et al*. (2002), Biffa *et al*. (2005), and Abera *et al*. (2012) who reported 38.2% in Adami-Tulu in central Ethiopia, 34.9% in Southern Ethiopia, 37.1% in Shashemene in southern Ethiopia, respectively. However, the present finding is relatively lower than the report of Mungube *et al*. (2004), Sori *et al*. (2005), Bedada and Hiko (2011) and Bedane *et al*. (2012) who recorded 46.6% from central highlands of Ethiopia, 52.8% from Sebeta, 66.1% from Assela in south eastern Ethiopia, 59.1% from Yabello, southern Ethiopia, respectively. Moreover, Abdelrahim *et al.* (1990) found a prevalence of 45.8% in Sudan, Kivaria *et al*. (2004) reported a prevalence of 90.3% in Tanzania and Radostits *et al.* (2000) described the prevalence of mastitis to be around 50% in cows in most countries irrespective of the causative agent. On the other hand, the result of the present study is higher than the prevalence of 31.7% reported by Berhanu (1997) in Eastern Harerghe and 28.2% in Bahir Dar by Bitew *et al*. (2010). Mastitis is a complex disease, and the difference in the prevalence reports of mastitis in the present study and other reports could be attributable to differences in breeds of targeted cows, farm management practices, level of production and differences in study methods and materials employed by the investigators. The differences in prevalence are most likely due to individual cow factors that considerably influence mastitis prevalence (Mekonnen and Tesfaye, 2010).

The frequencies of clinical and subclinical mastitis are highly esteemed parameters in the evaluation of the health of the bovine mammary gland (Fonseca & Santos, 2001). The present study revealed that prevalence of clinical and sub clinical mastitis at cow level was 4.95% and 31.77%, respectively. This result is comparable with the finding of Benta & Habtamu (2011) and Moges *et al*. (2011) who reported 5.3% of clinical and 31.67% of subclinical mastitis at cow level, respectively. Moreover, Gizat *et al*. (2007) reported the prevalence of clinical and subclinical mastitis at the rate of 3.9 and 34.4%, respectively. However, higher prevalence rates of clinical mastitis (Kerro and Tareke, 2003 (37.1%); Almaw *et al*., 2009; (25.22%); Mekibib *et al*., 2010 (22.4%) and Bedane *et al*., 2012 (21.1%)) and subclinical mastitis (Kerro and Tareke, 2003 (62.9%); Mekibib *et al*., 2010 (48.6%); Benta & Habtamu, 2011 (46.6%) and Tesfaye *et al*., 2012 (41.4%)) has been reported. The difference in prevalence of subclinical mastitis may be due to the different husbandry practices, diagnostic techniques, environmental conditions and immune status of animals. Since, environmental factors play a significant role, the prevalence of clinical and subclinical mastitis varies in dairy animals (Radostits *et al*., 2007).

In this study subclinical mastitis has been found to be higher than clinical mastitis. This could be attributed to ease of detection of clinical mastitis and treatment of only clinical cases. In most developing countries including Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Aarestrup *et al*., 1994). Moreover, subclinical mastitis has been reported to be higher than clinical mastitis owing to the defense mechanism of the udder, which reduces the severity of the disease (Hussein *et al*., 1997; Quinn *et al*., 2002; Mekonnen *et al*., 2005; Hundera *et al*., 2005). Because of its insidious nature, the subclinical mastitis might be among the causes of sub optimal milk production that is evident in many smallholder farms. According to Radostits *et al*. (2007), an infected cow and quarter show 30% and 15% reduction in milk yield, respectively. Moreover, farmers in Ethiopia are not well informed about the silent cases of mastitis (Karimuribo *et al*., 2006). Ethiopian farmers especially smallholders are not well informed about the invisible loss from sub clinical mastitis (Hussen *et al*., 1997) since dairying is mostly a side line business on these farms. A similar observation of the dominance of subclinical mastitis was observed by several studies (Workineh *et al*., 2002; Kerro and Tarek, 2003; Sori *et al*., 2011).

Overall quarter prevalence of 26.43% was recorded in the current study. The quarter prevalence of mastitis found in this study was comparable with the finding of Abera *et al*. (2010) in Adama, and Fadlelmoula *et al*. (2007) in Germany who reported the quarter

prevalence rate of 29% and 27.57%, respectively. However, the current report is lower than the report made by Mekibib *et al*. (2010) in Holeta, Bedane *et al*. (2012) in Yabello and Bachaya *et al*. (2011) in Pakistan, who reported 44.9%, 38.7%, and 35.25%, respectively. On the other hand, the present study is higher than the result of Kerro and Tareke (2003) from southern Ethiopia and Moges *et al*. (2011) from Gonder, who documented 18.7% and 12.73%, respectively. Quarter level prevalence of clinical (2.28%) and sub-clinical (24.15%) were observed which is in close agreement with the finding of Bitew *et al*. (2010) and Bedane *et al*. (2012) who recorded prevalence of clinical (1.9%) and subclinical (25.3%) mastitis at quarter level. However, it is lower than the previous report of Kerro and Tareke (2003) who reported the prevalence of clinical and subclinical mastitis to be 39.2, 60.8%, respectively. The difference in quarter wise prevalence of clinical and subclinical mastitis observed in the current study and previous studies may be due to the difference in breeds of animals, immune status, and managemental practices. The blind teat accounted 1.3%, which may be an indication of serious mastitis problem on the herd and lack of screening tests and treatment of subclinical mastitis, and inadequate follow up chronic mastitis were considered to be the major reason for the development of quarter blindness (Biffa, 2005). As compared to the others the right rear quarters were affected with the highest infection rate (27.15%). The left rear quarters were the second with an infection rate of 25.67%. This might be due to the high production capacity of the hind quarters followed with relaxed teat sphincters (Radostitis and Blood, 1994) and the high chance of getting fecal and environmental contamination (Sori *et al*., 2005). These results are supported by various other workers who also reported an increased prevalence of mastitis in rear quarters (Zeryehun *et al*., 2013; Zenebe *et al*., 2014).

The prevalence of mastitis was significantly associated with age and parity ($p < 0.05$). Thus, prevalence was relatively higher in adult cows (OR = 1.784), multiparous (OR = 1.320) than those corresponding animals. Significant association of age and parity with mastitis was reported by other authors (Abera *et al*., 2010; Moges *et al*., 2011; Zeryehun *et al*., 2013). Cows with many calves (>7) have about 13 times greater risk (62.9%) of developing an udder infection than those with fewer (3) calves (11.3%) (Biffa *et al*., 2005). The increased prevalence of mastitis in older animals in this study can be related to increased susceptibility of pathogenic organisms in udder relaxed sphincter muscles of teats. According to Erskine *et al*. (2002), primiparous cows have more effective defense mechanism than multiparous cows.

The prevalence of mastitis varied significantly ($p<0.001$) among breeds, where higher prevalence was recorded in the cross (68.42%) than Zebu (28.89%). Cross breed cows had shown to have a significant effect ($p<0.001$, OR=5.820, 95% CI = 3.248, 10.430) on the prevalence of bovine mastitis. The observed higher prevalence of mastitis in cross compared to local cows is in agreement with the findings of Biffa *et al*. (2005), Girma (2002) and Biru (1989). As stated in Radostits *et al*. (2007) this may be associated with differences in anatomical and physiological characteristics of the mammary gland, as well as high milk yielding of the cows. Furthermore, increase in milk yield from genetic selection may be accompanied in genetic susceptibility to mastitis. Therefore, the lower prevalence in local zebu cows in this study could be associated with the difference in genetic controlled physical barriers like streak canal sphincter muscle, keratin in the teat canal or shape of teat end where pointed teat ends are prone to the lesion. In addition to the physical barrier, the difference in the occurrence of mastitis in these breeds could arise from the difference in cellular immunity.

The finding of this study also showed the higher prevalence rate of mastitis in early (47.06%) and late (50.83%) stages of lactation as compared to mid (24.49%) stage of lactation with significant association ($p < 0.001$) with mastitis. Early and late-stage of lactation had shown to have a significant effect (early-stage, $p<0.001$, OR=3.021, 95% CI = 1.617, 5.647; late-stage, $P<0.001$, OR=3.280, 95% CI=1.931, 5.572) on the prevalence of bovine mastitis when compared to mid-lactation stage. This finding is in agreement with the previous results of Kerro and Tareke (2003) and Biffa *et al*. (2005) and Abera *et al*. (2012) who reported a high prevalence of mastitis in the early and late-stage of lactation. The udder is most sensitive to acute clinical mastitis and subclinical mastitis during the period after the calving, whereas chronic mastitis, most often subclinical, is more frequent later during the lactation. On the other hand, cows also get a natural high cell count towards the end of lactation because of reduced milk production (Andersson *et al*., 2011).

Cows with the previous history of mastitis had higher mastitis prevalence ($P<0.001$) compared to cows with no previous history of mastitis. The multiple logistic regression analysis also revealed a significant association of previous mastitis record (OR=2.452, 95%CI= 1.282, 4.688, $p<0.01$) with the prevalence of mastitis. Cows with the previous history of mastitis were found more likely to be mastitic. This observation is supported by the findings of Biffa *et al*. (2005) and Abera *et al*. (2012) who disclosed similar reports. This finding suggests that treatment of cows for mastitis may not be effective in eliminating the pathogens and the disease may be carried over from previous lactations to next lactation. Also, there are reports of antimicrobial resistance among pathogens which cause mastitis in Ethiopia (Abera *et al*., 2010).

Cows that were not treated during dry period were more affected than those treated and significantly associated with the prevalence of mastitis ($p<0.05$). This could be associated with the low bactericidal and bacteriostatic quality of milk during the dry period. Moreover, the capacity of the quarter to provide phagocytic and bactericidal activity generally diminishes during the dry period (Paape and Miller, 1996). Studies show that teat dipping after milking reduces the spread of infection from cow to cow, while dry cow therapy reduces the reservoir, which in turn further reduce the teats from bacterial exposure (Smith & Hogan, 1995). During the dry period, a keratin protein substance is produced to protect the streak canal (Eberthart, 1986).

The result of the present study also revealed the higher prevalence of mastitis (40.44%) in cows with poor udder/teat hygiene as compared to cows with good udder hygiene (18.46%). Odds ratio indicated that cows with poor udder hygiene were 13.39 times more likely to be exposed to mastitis than those with good udder hygiene. The current result is in agreement with the finding of Fentaye *et al*. (2014). Sanitary milking habits are important to avoid the spreading of bacteria or their proliferation. Milking practice had a significant influence on the prevalence of bovine mastitis. In this study, owners who didn't wash teats before and after milking found to have a high prevalence of mastitis than owners who used to. Improper washing of hands and teats before milking and use of one towel for each cow contribute to the prevalence of mastitis (Byarugaba *et al*., 2008). Radostitis *et al.* (2007) documented that udder preparation both before and after milking influence the rate of mastitis. Inadequate sanitation of dairy environment and lack of proper attention to the health of mammary gland were important factors contributing to the prevalence of mastitis (Musse *et al*., 2014).

Prevalence of mastitis was higher in those farms with poor drainage/slope for the stable area with significant association obtained between mastitis prevalence and drainage system which is in agreement with a report made by Abera *et al*. (2012). Poor drainage/slope of the stable area results accumulation of liquid such as urine and water used for cleaning of udders during milking. The liquid material mixed with the feces of the cows that led to dirty udder and teat. The environmental bacteria such as *E. coli* and other got access to enter trough teat canal and result in infection (Tesfaye *et al*., 2012).

Cows kept in houses with soil floor had a higher prevalence than cows managed on the concrete floor. Houses with soil floor increased the risk of mastitis. The association between soil floor and high prevalence of mastitis recorded in our study is consistent with the findings of Abera *et al*. (2010). This might be due to the favorable environment created for survival and multiplication of bacterial pathogens. Earlier works implicated poor barn hygiene to have a high prevalence of mastitis (Sori *et al*., 2005).

A significantly greater prevalence of mastitis was observed for cows maintained in zero grazing system (OR=1.892, 95%CI= 1.022, 3.501, $p<0.05$) than free grazers. Some authors affirmed that cows raised intensively are more susceptible to the development of intramammary infections through the greater concentration of animals and exposure to organic matter and pathogenic microorganisms (Kalmus *et al*., 2006).

The result obtained from bacteriological analysis of the samples revealed the predominant organisms isolated from bovine mastitis found to be *Staphylococcus aureus* (33.24%) followed by *Streptococcus agalactiae* (22.25%). *Staphylococci* and *Streptococci* species together accounted for 83.15% of the total isolates, while *Staphylococci* alone were 52.53% of the isolates. These bacteria were implicated as the most frequently isolated from mastitic milk in Ethiopia: *Staphylococci* and *Streptococci* species accounted for 73.5% (Workineh *et al*., 2002), 63.0% (Kerro and Tareke, 2003), 73.2% (Sori *et al*., 2005), 89.0% (Almaw *et al*., 2008), 57.2% (Mekonnen and Tesfaye, 2010) and 79.3% (Tesfaye *et al*., 2012) of the total isolates of bacteria from mastitic milk. The high prevalence of *Staphylococci* and *Streptococci* may be partly explained by presence of these agents on the skin and mucus membranes of various parts of the animal body (Carter and Wise, 2004; Quinn *et al*., 2004) and their contagious nature, especially *Staphylococcus aureus* and *Streptococcus agalactiae* (Radostits *et al*., 2007).

Moreover, the predominance and primary role of *Staphylococcus aureus* isolates in bovine mastitis has also been reported in other studies (Mekbib *et al*., 2010; Gitau *et al*., 2011; Asamenew *et al*., 2013; Alekish *et al*., 2013). Detection of *Staphylococcus aureus* at highest frequency in the current study could be due to its ability to evade and influence the host immune system by production of various enzymes and toxins that cause damage to mammary tissue and allow tissue invasion. In addition, *Staphylococcus aureus* is capable of surviving in the keratin of the teat canal of healthy cows and to confront phagocytosis. Furthermore, many *Staphylococcus aureus* strains can resist antibiotic therapy by the production of beta-lactamase, an enzyme that inactivates penicillin, and closely related antibiotics. Probably around 50% of mastitis caused by *Staphylococcus aureus* strains produce beta-lactamase and there is evidence that these strains are more difficult to cure with all antibiotics (Levy, 1998; Martin and Andrew, 2004). Furthermore, the finding of a higher proportion of *Staphylococcus* species might be due to lack of effective udder washing and drying, post-milking teat dip and drying and hand washing (Radostits *et al*., 1994). It is also attributed to the wide distribution of the

bacteria on the skin of teats and udder. The *staphylococci* have adapted to survive in the udder; they usually establish chronic, subclinical, infection and are shed in the milk which serves as a source of infection for other health cows during the milking process (Radostits *et al*., 2007).

In this study, *Streptococcus* species accounted for 31.87% of the total isolates next to *Staphylococcus* species. This finding was in agreement with Almaw *et al*. (2008), Mekonnen and Tesfaye, (2010) and Tesfaye *et al*. (2012). The relatively lower prevalence compared to *Staphylococcus* species might be due to their ready response to treatment as a cause of mastitis. The reason for the lower isolation rate of *Streptococcus* species is wide spread usage of penicillin for the treatment of mastitis because penicillin is effective antibiotic against this species of bacteria (Fantaye *et al*., 2014).

Coliforms (*Escherichia coli* and *Klebsiella pneumonia*) were the third most commonly isolated bacteria (10.17%) after *Staphylococci* and *Streptococci* which are in close agreement with the report of Kerro and Tareke (2003), Mekonnen and Tesfaye (2010) and Asamenew *et al*. (2013). Because these bacteria are environmental pathogens, their occurrence may be associated with poor quality management of housing, bedding and general lack of farm cleanliness and sanitation as they are commonly found in manure, soil and contaminated water (Hogeveen, 2005; Radostits *et al*., 2007).

The present study disclosed that prevalence of *Corynebacterium bovis* was 4.4% which was in close agreement with the report of Langoni *et al*. (2011). The natural habitat of *Corynebacterium bovis* is teat canal of cows (Quinn *et al*., 2004). Blowey and Edmondson (2010) reported the association of *Corynebacterium bovis* with poor post milking teat disinfection. Moreover, the current study revealed the prevalence of *Pseudomonas aeruginosa* at a rate of 2.2% that concord with the finding of Tesfaye *et al*. (2013). *Pseudomonas aeruginosa* is associated with contaminated water sources and can cause severe mastitis (Blowey and Edmondson, 2010).

V. Conclusion

The present study revealed that bovine mastitis is prevalent in smallholder dairy farms in the study area, and further confirms that the subclinical form is the most prevalent. The predominant bacterial species isolated in the study area were *Staphylococci* followed by *Streptococci species and* coliforms. Age, parity, breed, stage of lactation, previous mastitis record, udder hygiene, drainage/slope, floor type and grazing system were found to be risk factors significantly related to mastitis prevalence. Determination of mastitis causing organisms and putative potential risk factors is vital not only for the choice of treatment of the affected animals but also for devising effective management practices against associated risk factors. Bovine mastitis is prevalent in the study area and undoubtedly will hurt productivity of dairy industry and hence warrants serious attention. Regular screening for the detection of subclinical mastitis and proper treatment of the clinical cases, good milking hygiene as well as appropriate treatment of cows during dry and lactation period should be practiced.

Conflict of Interests: The authors have not declared any conflict of interests.

Acknowledgments

The author would like to acknowledge Madda Walabu University research, community engagement and technology transfer vice president office for logistic and financial support. Special thanks are extended to Sinana districts Agricultural office staff, cattle owners and respondents for their cooperation and support during data collection.

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The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
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Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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ISSN 9755896