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Biological Science

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Highlights

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Discovering Thoughts, Inventing Future

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Evaluation of Anti Bacterial Activity of Fresh Plant Extracts on Salmonella, Shigella and E.Coli

By Tegegne Bayih & Abdulahi Abdulhakim
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Abstract- The study conducted to investigate significant anti bacterial activity of egarlic (Alliums sativum L.) peach leave (prunus persica L) and root of ginger (zingiber officinale L) extract to bacteria like salmonella, shigella and E.coli. The objective of the study was to evaluate the antimicrobial activity of fresh botanicals extracts. Fresh botanical plant parts such as leave of prunus persica, root of zingiber officinale L and edible part Alliums sativum L were used for the investigation and fresh parts of plants were crushed by mortar and pestle to extract aqueous. Botanical extract that selected for this study had significantly inhibit for the formation colonies of bacteria spp such as Salmonella typhimurium, Shigella dysentery and Escherichia coli. All Treatments of the present study had showed high toxicity or inhibition of bacteria spp. Colonies when compared with negative treatment. Some aqueous extractions had no significance difference for inhibition of colonies when contrast with positive control (Ampicillin). Among treatments there was high significance difference ($p<0.001$) of capability of toxicity against bacteria cells. Finally, Escherichia coli spp had best resistance to extraction when compared with other bacteria spp. They were involved in the present investigation. In contrary, Shigella dysentery was more susceptible to the extraction.

Keywords: *alliums sativum, prunus persica, zingiber officinale, e.coli, salmonella, shigella.*

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Evaluation of Anti Bacterial Activity of Fresh Plant Extracts on Salmonella, Shigella and E.Coli

Tegegne Bayih [α] & Abdulahi Abdulhakim [σ]

Abstract- The study conducted to investigate significant anti bacterial activity of egarlic (Alliums sativum L.) peach leave (prunus persica L) and root of ginger (zingiber officinale L) extract to bacteria like salmonella, shigella and E.coli. The objective of the study was to evaluate the antimicrobial activity of fresh botanicals extracts. Fresh botanical plant parts such as leave of prunus persica, root of zingiber officinale L and edible part Alliums sativum L were used for the investigation and fresh parts of plants were crushed by mortar and pestle to extract aqueous. Botanical extract that selected for this study had significantly inhibit for the formation colonies of bacteria spp such as Salmonella typhimurium, Shigella dysentery and Escherichia coli. All Treatments of the present study had showed high toxicity or inhibition of bacteria spp. Colonies when compared with negative treatment. Some aqueous extractions had no significance difference for inhibition of colonies when contrast with positive control (Ampicillin). Among treatments there was high significance difference ($p<0.001$) of capability of toxicity against bacteria cells. Finally, Escherichia coli spp had best resistance to extraction when compared with other bacteria spp. They were involved in the present investigation. In contrary, Shigella dysentery was more susceptible to the extraction.

Keywords: alliums sativum, prunus persica, zingiber officinale, e.coli, salmonella, shigella.

I. Introduction

Medicinal plants are local heritage with global importance. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Garlic (*Allium sativum L*) is an herb used widely as a flavoring in cooking has also been used as a medicine throughout ancient and modern history to prevent and treat wide range of conditions and diseases. Garlic also thought to help reduce high cholesterol and elevated blood pressure. Note that garlic also contains anti oxidants that help to remove environmental toxins land west product of normal body pressure in the blood. The oils that extracted from the species and herbs are found to be effective in killing bacteria, viruses and others [medical center report 1995].

Garlic is used for many conditions related to heart and blood system these conditions include high blood pressure, high cholesterol coronary heart disease and hardening of arteries. These were supported by science and also ability to combat the common cold [Desta B, 1994].

Zingiber officinale L (ginger), a horizontal, branched, fleshy, aromatic white to yellow coloured perennial herb with leafy stem up to 60 cm, has long been used in the field of medicine. It belongs to the family Zingerberaceae. Its leaves are narrow being 20 cm long and 1.5-2cm wide. Dense spiked, yellow green with purple ending flowers are seen [Ross, I.A. 2005)]. The rhizome is rich in secondary metabolites such as phenolic compounds (gingerol, paradol and shogaoal), volatile sesquiterpenes (zingiberene and bisabolene) and monoterpenoids (curcumene and citral) Ginger has been widely used all over the world in ayurvedic medicine, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases [Ali et ai, 2008]. It has direct anti-microbial activity and thus can be used in treatment of bacterial infections [Tan Bkh and Vanitha J. 2004]. Ginger are relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also been "Generally Recognized as Safe".

Prunus persica (Aaru) belongs to the family Rosaceae is a deciduous tree up to 10 m high commonly cultivated for edible fruits from sub-Himalayan region up to 2400 m [Raturi R et ai 2011]. Peach (Prunus persica L.), from the family Rosaceae, are one of the most widely consumed fruits during the summer season. The fruits, with somewhat sour and astringent taste, are low in caloric content but have high nutritive value. They contain natural sugars (sucrose, glucose and fructose), organic acids (citric acid and malic acid), fiber (pectin), tannins, and no saturated fat [Anwar F et al. 2014].

Infectious rate by micro organism in developing countries is remaining high. Diseases continue to be a problem where nutrition, sanitary conditions are pure and emerging disease is more dangerous for such population.

In Ethiopia studies indicate that common bacterial infection is salmonella, E.coli and shigella. The mode of transmit ion is through water and food

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contamination. In urban & ruler area of Ethiopia these sanitary condition are poor. This condition exposed the population by those strain of bacteria. Disease due to food borne pathogens also remains a problem largely by consumption of improperly processed and stored food. Understanding the source of contamination and developing ways of limits the growth of pathogen is the role of education [WHO, 2001].

The study is conducted to minimize the disease causing bacteria. Typically salmonella, shigella and E.coli because of that the bacteria are the most problems of our food poising and causing disease. And the main problem of this study was the result of antibacterial activity of garlic (Allium sativum L) on bacterial growth. Ethiopia has various topographic land forms and biodiversity of plant species are applicable for traditional medicine making purpose among these traditional plants can be widely used are garlic is mentioned. But the effectiveness of the garlic has not been scientifically evaluated. This kind of research contributes to scientifically evaluate and increase the user garlic to reduce infection [Jonker D et al .1999].

Many studies have been conducted on different plant species of traditional medicine and in Ethiopia especially, garlic has been considered to be wonder drug for treatment and prevention of variety of disease but for generation people have information of its medicinal value through garlic has been widely used as antibiotic and treatment of cardiovascular disease, bites, tumors, ulcer, wounds, headacks, cancers, measles and many mores [Jonker D et al .1999].

Micro organisms are pathogenic organism vaccine, antibiotic and many other advances have lessened the impact of pathogens in the developed world. But microbial Infection in developing countries is high new illness caused by micro organisms continue to emerge and known pathogens becoming resistant, however when we observe specifically its difficult [medical center report 1995].

Salmonella: salmonella infection is common bacterial disease that affects the intestinal tract. Typically it live animal and human intestinal and are shed through feces. Humans infected most frequently through contaminated water and food. Salmonella is the second most frequent bacterial infection disease in the world. Infection with salmonella includes fever, abdominal pain and diarrhea.

E.coli bacteria normally live in the intestine of animals. Most of E.coli are harm less and actually important part of healthy human intestinal tract. However, some E.coli are pathogenic meaning they can cause illness either diarrhea or illness outside of intestinal tract. Shigella also a intestinal disease causing by shigellos. the main sign is diarrhea, fever, abdominal pain and malaise. But it is easily treated bacterial infection. [medical center report 1995]. this study has the following reasons: given appropriate treatment: because of the plant by nature rice in different nutrients and ability of antimicrobial activity then give effective treatment. It's the best way for the discoveries of other medical plants: because of Ethiopia have a large biodiversity of floras species. From thus large groups of flora there are numbers of plants have medical properties but they are not properly known. Therefore Ethiopia flora needs an extra investigation for discoveries and effective form of treatment from these species. Significance for society in order to give awareness by easy way of protecting health by using botanical plants to eradicate the disease causing by bacterial species.

Table 1: List of Botanicals and Parts used in Investigation

No.	Scientific Name	Common Name	Parts Used	Local Name
1	Prunus Persica L	Peach	Fresh Leave	Kock
2	Zingiber Officinale L	Ginger	Fresh Root Part	Jinjible
3	Allium Sativum L	Garlic	Edible Part	Nech Shinkurit

II. Materials and Methods

a) Study Area

The study was conducted in Hawassa university main campus, which is found in SNNPE Regional State, Hawassa city is found at a distance of 273 kilometer from Addis Ababa in the south direction. 7°3′N 38°28′E / 7.050°N 38.467°E and an elevation of 1708 meters above sea level. Hawassa city has a tropical savanna climate though it borders on a subtropical highland climate.

b) Plants Collection

Botanical plants were distributed in the rural and urban areas of the world. By using its botanical identities about plants sample for this study was collected from the market around 10k.m from Hawassa University Main Campus. To conduct this study the sample was taken from the healthy stem of garlic (*Allium sativium*), seed of peach leave (*prunus persica)* and root of ginger (*zingiber officinale).*

c) Preparation of Aqueous Plant Extracts

By using glove the fresh plant parts were collected from vicinity farm and 100 grams each plant part portion was chopped and cleaned. Cleaned parts were sterilized by immersing them up to 70% ethanol for two minute. Residual ethanol on surface was evaporated by air flow, followed by homogenized

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aseptically in sterile mortar and pestle. 5ml distilled water used to get enough aqueous solution. The homogeneity was then filtered by sterile cheese cloth to give a crude aqueous extracts of 10ml of each plant part was collected in sterile vial and stored in refrigerator until test of bacterial activity.

d) Source of Bacterial Strain

A Total of three gram-negative bacteria isolates species were selected for study. The isolates were obtained from the microbiology laboratory of Hawassa University. Bacterolligically the isolates were identified as salmonella, shigella, and E.coli by using standard procedure.

e) Media Preparation

In this study nutrient agar was formulated for the growth of three strains of tested bacteria that includes salmonella, shigella and E.coli. Nutrient agar media support the growth of total three microorganisms on agar. For this investigation (Four) agar media including 0.5ml 1ml, 1.5 ml 2ml, plant extracts aqueous and control group (positive and negative) were prepared for each strain of bacteria that was corresponding to the formulated test tubes.

f) Procedures and Anti-bacterial Test

The plants extract were taken from refrigerator and by using sterilized pipette it was spread in prepared separate media in different of concentration (0.5 ml, 1 ml, 1.5 ml 2ml of each plant extracts aqueous) and control group. The selected bacteria specimen dropped in to nutrient agar medium and incubated for 48hrs by 37 degree Celsius in the incubator.

g) Preparation of Colonies of Bacteria

After preparing suitable media agar add the inocula on the nutrient and put in incubator for 48 hrs to get Colonies. After days via counting using hand lens only 50 colonies of bacteria for each species were marked on the agar then plant extracts added to media agar which contain colonies put in incubator for 48 hrs and count how much is eradicated.

h) Data Analysis

All data were checked for normality before they were subjected to analysis. Data which lacked normality were transformed using appropriate transformations method. Data were analyzed with analysis of variance (ANOVA) using General Linear Model (GLM) in SAS software. Significant means were separated using Student-Newma Keuls (SNK) test.

III. Results

Table 2: Fresh Garlic (*Allium Sativium L)* aqueous extraction of toxicity with different volume of and mean of eradication of colonies formed by three species of bacteria

Treatment Doses	Mean ± SE Number of Colonies (within 48 hrs)		
	Salmonella Typhimurium	Shigella Dysentery	Escherichia Coli
0.5 ml	72.20±2.30 C	66.98±2.50 E	24.63±4.60 F
1 ml	76.15±1.54 C	60.25±4.23 E	42.00±3.52 G
1.5 ml	84.31±3.21 A	80.67± 3.12 A	60.54±4.02 H
2 ml	92.75±0.21 B	88.55±1.20 F	68.28± 1.20 J
Ampicillin (positive control)	98.56±0.10 B	96.69± 13.5 B	90.23± 1.26 B
Negative Control	0.00± 0.00D	0.00± 0.00 D	0.00± 0.00 D
P - Value	<0.001	<0.001	<0.001

Mean followed the same letters in column showed not significantly different using Student-Newman-Keuls (SNK) test (P<0.05).

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Table 3: Fresh Ginger (*Zingiber Officinale L)* aqueous extraction of toxicity with different volume of and mean of eradication of colonies formed by three species of bacteria

Treatment Doses	Mean ± SE Number Of Colonies (Within 48hrs)		
	Salmonella Typhimurium.	Shigella Dysentery	Escherichia Coli
0.5 ml	66.41±2.42 C	67.18±3.60 C	30.21±4.36 C
1 ml	69.12±1.24 C	60.31±4.01 C	45.25±3.69 E
1.5 ml	80.60±5.21 A	75.25±2.51 A	62.00±3.35 A
2 ml	94.50±0.23 B	87.10±2.21 E	65.00±1.25 A
Ampicillin (positive control)	98.00±0.01 B	96.30±0.13 B	92.31±0.65 B
Negative Control	0.00±0.00 D	0.00±0.00 D	0.00±0.00 D
P - Value	<0.001	<0.001	<0.001

Mean followed the same letters in column showed not significantly different using Student-Newman-Keuls (SNK) test (P<0.05).

Table 4: Fresh Peach Leaves (*Prunus Persica L*) aqueous extraction of toxicity with different volume of and mean of eradication of colonies formed by three species of bacteria

Treatment Doses	Mean ± SE Number Of Colonies (Within 48 Hrs)		
	Salmonella Typhimurium	Shigella Dysentery	Escherichia Coli
0.5 ml	52.71±4.42 C	56.38±3.73 C	20.00±4.10 C
1 ml	63.58±2.35 E	60.65±.3.12 C	32.21±3.34 E
1.5 ml	74.39±1.64 A	70.11±2.22 A	50.10±2.25 A
2 ml	82.34±1.22 B	78.01±2.01 A	60.00±2.89 E
Ampicillin (positive control)	98.10±0.01 B	96.27±0.21 B	90.51±2.10 B
Negative Control	0.00±0.00 D	0.00±0.00 D	0.00±0.00 D
P - Value	<0.001	<0.001	<0.001

Mean followed the same letters in column showed not significantly different using Student- Newman-Keuls (SNK) test (P<0.05).

IV. Discution

Table 1 showed that fresh garlic (*Allium sativium)* extraction toxicity against colonies of different bacteria species. Different dosage rates of Garlic (*Allium sativium)* aqueous extraction significantly eradicate colonies of salmonella, shigella and E.coli. As showed in Table 1 that there were high mean eradication of human pathogenic bacteria was recorded in the application high amount aqueous garlic (*Allium sativium)* extraction, for instance 2 ml aqueous extraction of this plant had better elimination of colonies on agar while compared with lowest milliliter aqueous extraction.

The preset study of three bacteria species revealed that they had their own resistance capability against extractions as per high to low amount. For example, in the highest and lowest application of extracts E coli had high mean resistance against (*Allium sativium* when it compared with salmonella, shigella, i.e. higher numbers of colonies were observed, on the contrary shigella had the lowest resisnce to the garlic (*Allium sativium)* which means shigella was more susceptible. There was high significance difference (p<0.0001) between treatments and ampicillin for the eradications of colonies except at high dosage rate of garlic was no significance difference to be the reduction of salmonella compared with Ampicillin

For the present investigation fresh ginger (*zingiber officinale)* aqueous extraction of antimicrobial activity had been evaluated which is written in table 2. More or less this botanical extraction had similar effect with garlic against for the formation of colony. Moreover there was no significance difference between high dose of ginger (*zingiber officinale)* and positive control. For instance, mean of 94.50% and 98.00% were recorded for ginger (*zingiber officinale)* and ampicillin respectively. The above percentage showed that Salmonella was highly susceptible to positive control and high dose of ginger (*zingiber officinale).* This study agreed with [Bkh and Vanitha J. 2004] ginger has direct anti-microbial

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activity and thus can be used in treatment of bacterial infections. Additionally agreement approved with [Ali et al 2008] Ginger (*Zingiber officinale*) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases.

Furthermore, as indicted in table 2 there was high mean reduction of bacteria colony formation had been observed due to application of treatments when compared with Negative control. High susceptibility of Salmonella spp And Shigella spp was observed at higher dosage (1.5 ml and 2ml) of ginger (*zingiber officinale)* aqueous extraction. In contrast, Ecoli spp had high resistance against the above mentioned botanical. Like garlic (*Allium sativium)* the extraction of ginger (*zingiber officinale)* had similar toxicity for the eradication of bacteria colony that grown on agar media. This is happened at low and high doses of treatments. The present study revealed that negative control of all treatments which mentioned in above tables showed there were no eradication colonies i.e. mean of 0.00% resistance was recorded.

Peach leaves (*prunus persica*) antimicrobial activity was also evaluated and it showed eradication of bacterial colonies at different dose. There was no significance difference in mean eradication of Salmonella typhimurium among all treatment especially at highest dose of (2 ml) aqueous extractions. For example, *Allium sativium, zingiber officinale and prunus persica* had showed mean of 92.75%, 94.50% and 92.34% respectively.

According to the researchers [Tuba Sevgi and Elif Demirkan.2017] peach (*prunus persica*) fruits contain phenolic additives which may show more or less antimicrobial effects. Depending on their antioxidant properties phenolic substances, which have effect mostly on color, flavor and durability of fruits and vegetables, are closely related with human health in terms of antimicrobial, anti-carcinogenic and anti-mutagenic activities. In the long term, bacterial resistance against antimicrobial agents may cause problems in fighting against several diseases. Therefore investigation of novel antimicrobial agents derived from new and natural sources have become important.

Additionally, the peach plant antimicrobial activity was investigated by the authors [Ved Prakash et al, 2017] and they revealed that the antibacterial activity of methanol, acetone and aqueous leaf extracts of *Prunus persica* was determined *in vitro* against medically important pathogens such as *Escherichia coli*, *Yersinia pestis, Bacillus cereus, Pseudomonas aeruginosa, Listeria monocytogenes* and *Staphylococcus aureus* following agar well diffusion method using different concentrations (25%, 50%, 75% and 100%). Results showed low to significant antibacterial activity against the mentioned bacterial species. Methanol leaf extract was found to be more effective against selected pathogenic bacteria as compared to acetone and aqueous leaf extracts. Furthermore, the leaf extracts inhibited gram-positive bacteria more efficiently than gram negative bacteria. The present study was targeted on fresh leave extracted and showed antimicrobial activity as indicated in the above tables.

On the other hand, this study was evaluated the toxicity of ginger against bacteria at different of doses of extraction and showed high eradication colonies at high dosage. The current study was in agreement with previously investigations. For example, ginger (*Zingiber officinale*) had some effect on pathogenic bacteria. The plant extracts were prepared by weighing the plant leaves and roots (20, 40, 60, 80 and 100 g) into 100 mls of water and ethanol (at g/100 ml) and grounded to determine the extract concentrations. Serial dilutions of the antibiotics used were prepared to determine the various antibiotic concentrations. The results obtained showed that ginger extract of both the plant and root showed the highest antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* while the three antibiotics used (chloramphenicol, ampicillin and tetracycline) were also active but at less extent compared to ginger extract. The concentration of the plant extract had significant effect on the zone of inhibition on both organisms [A. Sebiomo et al 2011].

In summary, the present study was basically focused on lonely evaluate fresh botanical extracts according to dosage rates. As indicated in the above table *Allium sativium, and zingiber officinale* had toxicity against bacteria cells or colonies at all dosages as compared with *prunus persica* and negative control. Moreover, *Allium sativium and zingiber officinale* botanicals had almost similar effect with positive control (Ampicillin) in the eradication of colonies. Generally all treatments at different dosage had high toxicity effect than negative control. The authors recommended that further studies should be forwarded to extract or isolate the exact composition several botanical plants and create awareness among societies as a number of medicinal plants should be taken as diets.

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References Références Referencias

1. Medical Center Report (1995), extracted from the species and herbs effective in killing bacteria. University of merray land.

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2. Desta B, 1994, Ehiopian traditional herbal drugs, part two. Anti fertility activity of 70 medicinal plant.
3. Ross, I. A. (2005) Medicinal Plants of the World. Humana Press, Totowa, New Jersey: 507-560.
4. Ali, B. H., Blunden, G., Tanira, M. O., Nemmar, A. (2008): Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A Review of recent research. Food Chem. Toxicol. 46, 409-420.
5. Tan B. K. H. and Vanitha J. (2004). Immunomodulatory and Antibacterial Effects of Some Traditional Chinese Medicinal Herbs: A Review. Curr. Med. Chem., 11(11):1423-1430.
6. Raturi R., Sati S. C., Singh H., Bahuguna P. and Badoni P. P. (2011) Chemical examination and anti-inflammatory activity of Prunus persica steam bark. International Journal of Pharmacy and Pharmaceutical Sciences, 3(5):315.
7. Anwar F., Manzoor M., Bukhari I. H. and Aladedunye F. (2014) Physico-chemical attribute of fruit seed oils from different varieties of peach and pulm. Journal of Advances in Biology, 4 (2): 385.
8. WHO legal status of traditional medicine and complementary/ alternative medicine: worldwide geneva 2001.
9. Jonker D., Van Den and Broek E., Van, (1999). Anti-bacterial effect of garlic. Lambert, J., Ethiopian traditional medicine and the bridge to better health.
10. Tuba Sevgi and Elif Demirkan. 2017. Antimicrobial activities of seed extracts of *Prunus Persica, Prunus Cerasus, Prunus Avium* and *Prunus Armeniaca.* J Biotechnol Biomater 2017, 7:1.
11. Ved Prakash, Shelly Rana and Anand Sagar*, (2017).* Studies on analysis of antibacterial and antioxidant activity of *prunus persica* (l.) batsch. I.J.S.N., VOL. 8 (1) 2017: 54-58.
12. A. Sebiomo, A. D. Awofodu, A. O. Awosanya, F. E. Awotona and A. J. Ajayi. 2011. Comparative studies of antibacterial effect of some antibiotics and ginger (Zingiber Officinale) on two pathogenic bacteria. J. Microbiol. Antimicrob. Vol. 3(1), PP. 18-22.

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Role of Chitosan in Induction of Defense Response against *Phomopsis vexans* and Augmentation of Growth and Yield of Eggplant

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Abstract- Phomopsis blight and fruit rot disease caused by the fungus Phomopsis vexans is one of the most devastating diseases of eggplant that causes great economic losses especially in tropical and subtropical climates. An experiment was conducted to evaluate the antifungal properties of chitosan against P. vexans by inducing defense-related enzymes and increase the growth and yield contributing characters of eggplant. P. vexans isolate VS-2 was identified as most virulent against eggplant by pathogenicity test. The concentrations of chitosan 0, 0.1, 0.2, 0.3, 0.4 and 0.5% were used to control the fungus in in-vitro trial. There was an effect on mycelial growth reduction of P. vexans by chitosan. The highest mycelial growth reduction was found with 0.5% chitosan at seven days after incubation. Consequently, seeds were treated with chitosan @ 0.5% concentration. Chitosan enhanced the germination percentage and seedling growth such as shoot length, root length, fresh weight and dry weight and reduced post-emergence seedling mortality.

Keywords: *chitosan, defense, eggplant, growth, phomopsis blight and fruit rot, P. vexans, yield.*

GJSFR-C Classification: *FOR Code: 060799*

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Role of Chitosan in Induction of Defense Response against *Phomopsis vexans* and Augmentation of Growth and Yield of Eggplant

Rayhanur Jannat [α], Milon Shaha [σ], M. Tanbir Rubayet [ρ] & Salma Sultana [ω]

Abstract- Phomopsis blight and fruit rot disease caused by the fungus *Phomopsis vexans* is one of the most devastating diseases of eggplant that causes great economic losses especially in tropical and subtropical climates. An experiment was conducted to evaluate the antifungal properties of chitosan against *P. vexans* by inducing defense-related enzymes and increase the growth and yield contributing characters of eggplant. *P. vexans* isolate VS-2 was identified as most virulent against eggplant by pathogenicity test. The concentrations of chitosan 0, 0.1, 0.2, 0.3, 0.4 and 0.5% were used to control the fungus in *in-vitro* trial. There was an effect on mycelial growth reduction of *P. vexans* by chitosan. The highest mycelial growth reduction was found with 0.5% chitosan at seven days after incubation. Consequently, seeds were treated with chitosan @ 0.5% concentration. Chitosan enhanced the germination percentage and seedling growth such as shoot length, root length, fresh weight and dry weight and reduced post-emergence seedling mortality. In the field condition, chitosan reduced leaf blight and fruit rot disease incidence and severity, simultaneously increased plant height and number of branching in eggplant after certain maturity. It was also found to increase the yield and yield contributing characters of eggplant. The defense enzymes catalase (CAT) and peroxidase (POD) activities were also increased several folds by this elicitor. Chitosan contains antifungal properties against *P. vexans* as well as growth promoting substances to induce resistance of eggplant.

Keywords: chitosan, defense, eggplant, growth, phomopsis blight and fruit rot, P. vexans, yield.

I. Introduction

Eggplant (*Solanum melongena* L.) belongs to the family Solanaceae is a popular and widely grown year-round vegetable of Bangladesh. In Bangladesh, eggplant is the second most important vegetable crop with respect to total acreage (50181.02 ha) and production (475,000 MT annually) (BBS, 2017). Eggplant suffers heavy yield losses due to many diseases like bacterial wilt, little leaf, Phomopsis blight and fruit rot, Verticillium wilt, Sclerotinia blight, Fusarium wilt, root-knot and leaf spots, etc. Among them, Phomopsis blight and fruit rot caused by *Phomopsis vexans* have been treated as one of the constraints to eggplant cultivation in the country (Khan, 1999). This disease appears as damping off, tip over and seedling blight in the nursery and fruit rot in the harvesting crop (Singh, 1992).

Seed is the infection source of *P. vexans* and may serve as a substrate for pathogen survival (Pan and Acharya, 1995). The pathogens remain on the seed coat and the cotyledons of eggplant seeds which causes various degrees of seed discoloration and tiny black pycnidial bodies (Karuna *et al.*, 1994). Fresh seeds can be separated easily by discarding the infected, abnormal and discolored seed.

Nowadays new approaches and practices are being developed for sustainable crops and vegetable production in Bangladesh. Chitosan is one of the most abundant natural amino polysaccharides extracted from the exoskeleton of crustaceans, insect, fungal cell walls, etc. Chitosan has a wide variety of applications in agricultural and biotechnological industries (Brine *et al*., 1992; Majeti and Kumar, 2000). The antimicrobial activity of chitosan was recognized and considered as important natural properties which can be used directly for plant disease suppression (Zhao and Xia, 2006).

Management of many fungal pathogens in different pathosystems through the application of chitosan is well documented (Abd-El-Kareem *et al*., 2006; Chittenden and Singh, 2009; El-Mohamedy *et al*., 2014).

Defense mechanisms in plant has been accelerated by using chitosan. Ortega-Ortiz *et al.,* (2007) reported that chitosan had increased catalase (CAT) and peroxidase (POD) enzymes activity in *Lycopersicon esculentum*. An induction of resistance in plants by application of elicitor (chitosan) is becoming a promising approach for management of plant diseases. The introduction of chitosan into agricultural practice could minimize the scope of chemical control, thus contributing to the development of sustainable agriculture. However, there have been no published reports on induction of resistance in eggplant against *P. vexans* by chitosan. Therefore, the present experiment was designed to investigate the role of chitosan against *P. vexans* in inducing biochemical defense enzyme

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(Catalase and peroxidase) activation and increase the growth and yield of eggplant.

II. Materials and Methods

a) Experimental Site and Plant Material

The experiment was conducted in the Plant Pathology laboratory and research field in the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. Seeds of eggplant variety BARI Begun 4 (Kajla) were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Eggplant seedlings were grown in a fertile and irrigated plot an open ambient climate and net house. Leaves were collected from uniformly grown plants at seedling stage for using in enzyme extraction.

b) Isolation and Identification of P. vexans

The fungus was isolated from infected eggplants following the tissue planting method (Mian, 1995). The fungal colonies were grown on Potato Dextrose Agar (PDA) and identified by following standard key (Barneet and Hunter, 1980). The pure culture of *P. vexans* isolates was named individually with English capital letter and numerical number codes then preserved by using PDA slants at 10°C in the refrigerator as a stock culture for further study.

c) Preparation of Spore Suspension of P. vexans

The ten-days-old culture was grown on PDA plate and flooded with 10 ml of sterilized distilled water. Pycnidia and conidia along with mycelial mass were separated from the substratum by scrapping with a narrow edge glass slide (Jahanara *et al*., 2018) and blended in an electric blender at high speed for 5-7 minutes. The suspension was sieved through double layer cheesecloth to discard pycnidia and mycelial mass. The spores were counted under the compound microscope by using counting slide haemocytometer. The spore suspension was adjusted to $2x10^5$ ml^{-1} by adding sterilized distilled water (Ashrafuzzaman, 1976). Seeds were submerged in spore suspension with gentle stirring for 5 minutes, the wetted seeds were air dried in a sterilized cabinet, and then further treatments were done.

d) Pathogenicity Test for the Selection of Virulent Isolate of the Test Pathogen

Pathogenicity test was done against eggplant seedlings as well as detached fruits following the methods as stated by Jahanara *et al*., 2018. Four isolates of the test pathogen named as VS-1, VS-2, VS-3, and VS-4 were evaluated for their pathogenicity test in the tray under the shade condition. Tray was filled by sterilized soil. For inoculation purpose, seeds were treated with the spore suspension of the four isolates of *P. vexans* separately and sown in an individual tray. In control tray seeds were sown without any treatment. Disease development was observed timely and recorded at 20 days after sowing (DAS) to estimate the effect of the pathogen in causing pre-emergence and post-emergence seedling mortality. The causal agent of pre-emergence and post-emergence seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds and death seedlings respectively. For detached fruits, at first eggplant fruits were rinsed with sterilized water and air dried. The inoculation process was made by puncturing the tissue with small sterilized needle. Spore suspension (2×10^5 spore ml^{-1}) of *P. vexans* was sprayed over the eggplant fruits. The un-inoculated fruit was selected as control treatment. After inoculating, eggplant fruits were kept in a transparent polyethylene bag separately to maintain humidity for seven days at room temperature (25 ± 2°C). Disease symptoms and pycnidia formation were recorded, and percent disease index (PDI) was rated by following 0-4 scale, where 0=no visible sign or symptoms, 1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100% infection on eggplant fruits (Abraham *et al*., 1996).

e) In Vitro Evaluation of Chitosan for their Inhibitory Effect on the Radial Growth of P. vexans

A series of preliminary evaluation of chitosan was done by using the lower concentration such as 0.1, 0.2, 0.3, 0.4 and 0.5% on PDA plate against the *P. vexans*. Chitosan was added to conical flasks containing sterilized PDA before solidification and rotated gently then poured into sterilized petri dish (9 cm diameter). Individual plate was challenged with 7 days-old culture of the pathogen at the center by equal agar plug (5 mm in diameter), and incubated at room temperature (25 ± 2°C) for 72 hours. The colony diameter was measured when the control plate (without chitosan) reached full growth. The radial growths of *P. vexans* in three replicates were recorded separately, and their average data were taken. Based on the laboratory results, the most antagonistic effect of chitosan @ 0.5% conc. was selected for seed treating purpose. The percent inhibition of the radial growth was calculated as described by Jahanara *et al.,* (2018).

$$\% \text{ inhibition over control } = \frac{X-Y}{X} \times 100$$

Where,
The X= Mycelial growth of pathogen without chitosan (control),
Y = Mycelial growth of pathogen with chitosan

f) Seed Treatment with Chitosan

Chitosan was collected from Bangladesh Atomic Energy Commission, Dhaka, Bangladesh. It was derived from the shell of quick growing sea shrimp. The solution was extracted from sea shrimp, and then it was irradiated with γ-ray (20 kD). Seed treatment was done by following the standard procedure described by

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Mahdavi and Rahimi, 2013; Mishra *et al*., 2014. Seeds of eggplant were surface sterilized by immersion of 0.1% NaOCl, thoroughly rinsed in sterilized distilled water and were immersed into the 0.5% chitosan solution (pH 5.5-6.0). After gentle stirring submersed for 3 h, the wetted seeds were air dried in a sterilized cabinet and kept in a desiccator until use. For positive control Bavistin (0.1%) 50 WP was used, and there was no treatment in untreated control seeds. In a plastic tray, seeds were sown after required treatments for confirming the effect of chitosan on germination and post-emergence seedling mortality. Finally, data were recorded up to the complete emergence of the seedlings.

g) Land Preparation and Rising of Seedlings

The land was prepared with good tilth using a tractor driven disc plow and harrow. After land preparation the whole experimental area was divided into three blocks, representing three replications. The unit plot size was 3 m × 2 m where row to row distance 75 cm and plant to plant 75 cm. Distance between block to block was 1.0 m and that of plot to plot in a block was 0.5 m. Drains were made surrounding each unit plot and the excavated soil was used for raising plots 15 cm high from the general soil surface. Six different treatments were allotted randomly to each block. Thirty-five-days aged healthy eggplant seedlings of variety 'BARI Begun 4' were collected from the tray. Weeding, irrigation and other intercultural operations were done as and when necessary until the maturity of plants.

Treatments of the Experiment

The treatments of the field experiment were as follows:

T_1=Untreated seed (control-1)

T_2=Seed treated with *P. vexans* (control-2)

T_3=Seed treated with 0.5 % chitosan

T_4=Seed treated with *P. vexans* + 0.5% chitosan

T_5=Seed treated with 0.1% Bavistin

T_6=Seed treated with *P. vexans* + 0.1% Bavistin

h) Data Recording and Disease Assessment

Data were taken on germination percentage, mortality percentage, root length, shoot length, fresh seedling weight, seedling dry weight, enzyme activities (CAT, POD), plant height, number of branches, disease incidence, percent disease index (PDI), number of fruits, fruit weight and yield both in the absence and presence of *P. vexans*. Seedling growth was measured at 28 days after sowing (DAS). Plants were dried for dry weight measurement in an oven at 60°C for 3 days, and weight was evaluated for each treatment. Eggplants were observed regularly after transplanting of seedlings to record the incidence of post-emergence seedling mortality, Phomopsis leaf blight and fruit rot diseases on fruits. Disease incidence and PDI were recorded based on a scale of 0 to 4 as described by Abraham *et al*., (1996). Then, the following formulae were used for calculation (Rahman *et al*., 2013).

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

$$\text{Disease index} = \frac{\text{Summation of all rating of fruits observed}}{\text{Number of fruits observed} \times \text{Maximum rating}} \times 100$$

Percent disease index (PDI) was rated by following 0-4 scale, where 0=No visible sign or symptoms, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% infection on eggplant fruits.

i) Extraction of Protein and Defense Enzymes

The eggplant leaves were homogenized in 10 ml of chilled 0.1 M phosphate buffer (pH 7.0). The homogenate materials were centrifuged at 4°C for 30 min at 10000 rpm. After centrifugation, the supernatant portion was used as enzyme extract for the determination of enzyme activity.

j) Protein Assay

The protein content in the extracts was estimated by the dye-binding method of Bradfort (1976) using Bovine Serum Albumin (BSA) as standard.

k) Catalase (CAT) Activity Assay

The activity was assessed by measuring the rate of disappearance of H_2O_2 at 240 nm using a BioMate TM 3 spectrophotometer. The reaction mixture (2 ml) was consisting of 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.2 ml enzyme extract. Enzyme activity was calculated by using the molar extinction of co-efficient $36x10^3$ mM^{-1} min^{-1} and expressed as µmol H_2O_2 oxidized per g fresh weight per min (g^{-1} FW min^{-1}). One unit was defined as a changed in absorbance of 0.1 under the assay conditions (Cakmak and Marschner, 1992).

l) Peroxidase (POD) Assay

POD activity was assessed by following standard method (Chance and Maehly, 1955). The enzyme activity was determined by measuring with increasing in absorbance at 470 nm due to oxidation of guaiacol to tetraguaiacol. The reaction mixture was consisted of 20 mM guaiacol (0.5 ml), 0.1 mM acetate buffer (pH 5.0; 2.1 ml), 40 mM H_2O_2 (0.2 ml) and enzyme extract (0.2 ml) with a final volume of 3 ml. The linear portion of the activity curve was used to calculate enzyme activity. One POD unit was defined as the change of 1.0 absorbance unit per ml enzymatic extract and expressed as units of enzyme activity per g fresh weight per min (UA g^{-1} FW min^{-1}). One unit of enzyme activity was represented as the amount of enzyme catalyzing the oxidation of 1 µmol of guaiacol in 1 min.

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m) Experimental Design and Data Analysis

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Data were recorded on various parameters and analyzed statistically using the Statistix 10 statistical computer programme after proper transformation whenever necessary. The mean values were compared by following LSD (Least Significance Differences) test (Gomez and Gomez, 1984).

III. Results And Discussion

a) Pathogenicity Test for the Selection of Virulent Isolate of P. vexans

The pathogenicity tests of the randomly selected four isolates of the pathogen were done against eggplant seedlings as well as in the detached fresh fruits. Seedlings were grown in the plastic tray containing sterilized soil to select the most virulent isolate of the test pathogen for evaluation the effect of chitosan. The most virulent isolate was selected based on highest seedling mortality and disease symptoms and pycnidial formation of eggplant caused by the isolate. The *P. vexans* isolate VS-2 was appeared to be the most virulent isolate. Significantly, the highest 91.83% seedling mortality was caused by VS-2 isolate followed by VS-1 (70.93%) (Table 1 and Fig. I). On the contrary, significantly the lowest (53.90%) seedling mortality was observed with the isolates VS -3. No pre-emergence and post-emergence seedling mortality was observed in the control tray. All the tested isolates of *P. vexans* were found to be virulent and seriously causing seedling mortality. Pre-emergence seedling mortality was appeared higher at ranging from 42.17-78.53% than post-emergence mortality at ranging from 11.73-14.53%. Furthermore, every isolates were showed a pathogenic reaction to the detached eggplant fruits (Table 1 and Fig. 2). Among the eggplant fruits, which were inoculated with the isolate VS-2 showed highest PDI (88.90%). The virulence of the isolates in detached fruits supports the virulence of the same isolates in the earlier tray culture experiment. These results approved by the observation of Islam and Meah, 2011. The results of the present study indicated that all the isolates were pathogenic to eggplant seedling but the virulence of the isolates were variable. The VS-2 isolate of *P. vexans* was the most virulent to eggplant, and therefore, it was selected as test pathogen for inoculation in the field trial.

Table 1: Pathogenicity Test of *P. Vexans* Isolates on Seedlings and Fruits of Eggplant

P. vexans Isolates	Seedlings			Fruits PDI
	Pre-emergence mortality (%)	Post-emergence mortality (%)	Total mortality (%)	
VS-1	58.33	12.6	70.93 b	75.25 b
VS-2	78.53	13.3	91.83 a	88.90 a
VS-3	42.17	11.73	53. 90 d	59.56 d
VS-4	45.87	14.53	60.40 c	66.11 c
Untreated Control	0.00	0.00	0.00 e	0.00 e

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

Fig. 1: Pathogenicity Test of *P. vexans* Isolates against Eggplant Seedlings in Tray Culture.

Fig. 2: Pathogenicity Test of *P. vexans* Isolates on Eggplant Fruits.

b) Effect of Chitosan on the Mycelial Growth of P. vexans

The mycelial growth of most virulent isolate VS-2 of *P. vexans* in PDA plate were amended with five different concentrations viz, 0.1, 0.2, 0.3, 0.4, and 0.5% of chitosan (Table 2 and Fig. 3). All the selected concentrations of chitosan amended with PDA plate significantly reduced the mycelial growth of *P. vexans*

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over control PDA plate (without chitosan). But all the five concentrations of chitosan were significantly varied in reducing the mycelial growth of *P. vexans*. Significantly, the highest 84.58% reduction of the mycelial growth of *P. vexans* over the control PDA plate was observed at the highest 0.5% concentration of chitosan amended with PDA plate, followed by the second highest (73.75%) at 0.4% concentration of chitosan. On the other hand, significantly the lowest 22.5% reduction of the mycelial growth of *P. vexans* was observed at the lowest 0.1% concentration of chitosan amended with the PDA plate. Based on the *in-vitro* evaluation, 0.5% chitosan was selected for seed treatment. These results are supported by El-Mohamedy *et al*., 2014 who reported that chitosan applied at different concentrations (from 0.5 to 4 g/L) had decreased the mycelial growth of *Fusarium.* But the complete inhibition was obtained at the highest concentration @ 4 g/L.

Table 2: Effect of Chitosan on Mycelial Growth of *P. vexans*

Treatment	Average mycelial growth after 7 Days (mm)	% mycelial growth inhibition over control
No Chitosan (Control)	80.00 a	-
0.1% Chitosan	62.00 b	22.50
0.2% Chitosan	49.67 c	37.92
0.3% Chitosan	37.67 d	52.92
0.4% Chitosan	21.00 e	73.75
0.5% Chitosan	12.33 f	84.58

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

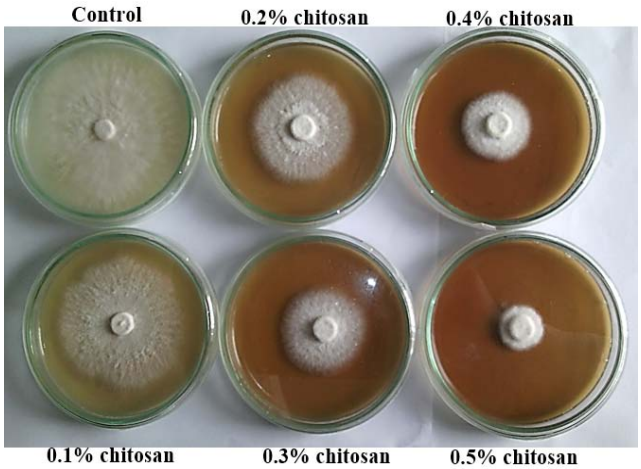

Fig. 3: Mycelial Growth Inhibition of *P. vexans* by Chitosan on PDA Plate.

c) *Effect of Chitosan and Bavistin on Germination Percentage and Post-emergence Seedling Mortality*

The germination percentage was increased in all treatments over the treatment T_2 where seeds treated with *P. vexans* (Table 3). The range of germination percentage was 67.33-93.33%. The highest (93.33%) germination was found in the treatment T_5 followed by the treatment T_3 (92.00%), T_4 (89.33%) and T_6 (88.67%) but these were statistically identical. In contrast, significantly the lowest germination (67.33%) was found in the treatment T_2 where seeds were treated with *P. vexans*. In case of seedling mortality, all treatments reduced post-emergence seedling mortality compared to the treatment T_2, where seeds were treated with *P. vexans* (Table 3). Significantly, the lowest (3.33%) post-emergence seedling mortality was found in the treatment T_3 followed by T_4 (4.44%), T_5 (5.56%), and T_6 (6.67%) but these were statistically identical. Chitosan increased 36.64% and 32.67% germination and decreased post-emergence seedling mortality by 85.73% and 80.97% in natural and inoculated condition of the pathogen respectively. This experiment showed that seed treatment with 0.5% chitosan was effectively to increase germination and control post-emergence seedling mortality of eggplant like Bavistin 50 WP. The similar results in increasing germination percentage and decreasing post-emergence seedling mortality with chitosan were reported by Mahdavi and Rahimi, 2013 and Jahanara *et al*., 2018.

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Table 3: Effect of Chitosan and Bavistin on Seed Germination and Post-emergence Seedling Mortality of Eggplant

Treatments	% Seedling Emergence	% Increase Over T_2	% Post Emergence Seedling Mortality	(%) Reduction Over T_2
T_1=Untreated Seed (Control-1)	80.00 b	18.82	16.67 b	28.55
T_2=Seed Treated With *P. vexans* (Control-2)	67.33 c	-	23.33 a	-
T_3=Seed Treated With 0.5% Chitosan	92.00 a	36.64	3.33 c	85.73
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	89.33 a	32.67	4.44 c	80.97
T_5=Seed Treated With 0.1% Bavistin	93.33 a	38.62	5.56 c	76.12
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	88.67 a	31.69	6.67 c	71.41

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

d) Effect of Chitosan and Bavistin on the Growth of Eggplant Seedlings

Growth promoting factors like shoot length, root length, fresh weight and dry weight were recorded randomly from five plants of each treatment at the seedling stage (28 DAS). Seed treatment with chitosan, as a result, increased the growth promoting components in comparison to the treatment T_2 where seeds were treated with *P. vexans* spore suspension (Table 4). The highest shoot length (6.16 cm), root length (4.54 cm), fresh weight (0.2728 g) and dry weight (0.0364 g) of seedlings were found in the treatment T_4, where seeds were treated with chitosan (0.5%) in the pathogen inoculated condition. On the other hand, the lowest shoot length (4.72 cm) and lowest fresh weight (0.058 g) were recorded in T_1, and lowest root length (2.44 cm) and dry weight (0.0082 g) were recorded in T_6. In seedling growth, the concentration of 0.5 % chitosan increased shoot length, root length, fresh weight and dry weight by 16.23, 29.96, 116.85, and 139.47% respectively in T_4 over T_2 treatment after 28 DAS. Plant height and number of branches were recorded randomly from three plants after certain maturity (75 days after transplanting) of eggplant in the field. Chitosan as seed treatment increased the plant height and number of branches in comparison to the treatment T_2, where seeds were treated with *P. vexans* (Table 5). The highest plant height (83.33 cm) and number of branches (23.00) were achieved by chitosan in the treatment T_3 followed by plant height (78.17 cm) and number of branches (22.00) in T_4 but no statistical difference was found among them. In contrast, the lowest plant height (50.40 cm) and number of branches (10.33) were recorded in the treatment T_2 where seeds were treated with *P. vexans*. These results are agreed with several investigators (Harada *et al*., 1995; Shao *et al*., 2005; Algam *et al*., 2010; Mahdavi and Rahimi, 2013; Mishra *et al*., 2014) where seed treatment with chitosan increased growth parameters of seedling as well as mature plant like shoot growth, branch length, node number per plant, seed yield, total root length per plant in different crops.

Table 4: Effect of Seed Treatment with Chitosan and Bavistin on Growth Parameters at the Seedling Stage (28 DAS) of Eggplant

Treatments	Shoot Length (cm)	Root Length (cm)	Fresh Weight of Seedling (g/plant)	Dry Weight of Seedling (g/plant)
T_1=Untreated Seed (Control-1)	4.72 c	3.00 b	0.0580 d	0.0104 d
T_2=Seed Treated With *P. vexans* (Control-2)	5.30 bc	3.18 b	0.1258 c	0.0152 c
T_3=Seed Treated With 0.5% Chitosan	5.92 ab	4.46 a	0.2302 b	0.0302 b
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	6.16 a	4.54 a	0.2728 a	0.0364 a
T_5=Seed Treated With 0.1% Bavistin	5.16 c	3.10 b	0.0658 d	0.0102 d
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	4.82 c	2.44 c	0.0746 d	0.0082 d

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

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Table 5: Effect of Chitosan and Bavistin on Plant Height and Number of Branches of Eggplant

Treatments	Plant Height (cm)	% Increase Over T_2	Number of Branching / Plant	% Increase Over T_2
T_1=Untreated Seed (Control-1)	57.17 c	13.43	11.67 c	12.97
T_2=Seed Treated With *P. vexans* (Control-2)	50.40 d	-	10.33 c	-
T_3=Seed Treated With 0.5% Chitosan	83.33 a	65.34	23.00 a	122.65
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	78.17 ab	55.10	22.00 a	112.97
T_5=Seed Treated With 0.1% Bavistin	78.50 ab	55.75	17.00 b	64.57
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	76.83 b	52.44	17.33 b	67.76

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

e) Effect of Chitosan and Bavistin on Enzyme Activity

Chitosan is known to have eliciting activities leading to a variety of defense responses in plants, in response to microbial infections to reduce the negative impact of diseases, on yield and quality of crops. Seed treatment with chitosan as an elicitor increased the CAT and POD activities (Table 6). The higher activities of CAT (256.88 μmol g^{-1} min^{-1}) and POD (0.9267 UA g^{-1} min^{-1}) were found in T4 followed by T3, CAT (235.28 μmol g^{-1} min^{-1}) and POD (0.7267 UA g^{-1} min^{-1}). Chitosan increased the CAT (113.48%) and POD (56.18%) activities in T4 over T2. The findings are in agreement with other investigators (Ortega-Ortiz *et al.,* 2007; Mandal, 2010; Mishra *et al.,* 2014) that chitosan treatment can cause induced resistance and increase enzyme activities in many plants. Chitosan also activates host defense genes leading to physical and biochemical changes in plant cells involved directly or indirectly in disease suppression.

Table 6: Effect of Chitosan on Enzyme Activity of Eggplant Leaves

Treatments	CAT (μmol g^{-1} min^{-1})	POD (UA g^{-1} min^{-1})
T_1=Untreated Seed (Control-1)	216.37 c	0.3833 d
T_2=Seed Treated With *P. vexans* (Control-2)	120.33 e	0.5933 c
T_3=Seed Treated With 0.5% Chitosan	235.28 b	0.7267 b
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	256.88 a	0.9267 a
T_5=Seed Treated With 0.1% Bavistin	175.56 d	0.4400 d
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	188.14 d	0.4067 d

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

f) Effect of Chitosan and Bavistin in Controlling Phomopsis Blight and Fruit rot of Eggplant in the Field

An attempt has been made for the management of Phomopsis leaf blight and fruit rot diseases of eggplant developed under inoculated condition through the application of chitosan. Phomopsis leaf blight and fruit rot disease incidence (DI) and percent disease index (PDI) were recorded from infected leaves and fruits at different growth stages from open field condition. Chitosan and fungicide treatments reduced leaf blight and fruit rot incidence and PDI in the field over T_2 where eggplant seeds were treated with *P. vexans* without using any biocontrol agent such as chitosan or any fungicide such as Bavistin (Table 7 and 8 and Fig. 4). The highest leaf blight (45.83%) and fruit rot (66.67%) incidence were found in treatment T_2 followed by leaf blight (37.5%) and fruit rot (45.83%) incidence in T_1. In contrast, treatment T_3 where seeds were treated with chitosan showed the lowest leaf blight (4.17%) and fruit rot (8.33%) incidence followed by T_4 and T_5 and no statistical difference was found among them. Similarly, significantly the highest leaf blight (46.67%) and fruit rot (61.67%) PDI were found in the treatment T_2 and the lowest leaf blight (10.00%) and fruit rot (8.33%) PDI were recorded in chitosan treated seed plot T_3. In the field, chitosan reduced disease incidence (leaf blight 81.82% and fruit rot 81.25%) and PDI (leaf blight 67.86% and fruit rot 78.38%) in T_4 over treatment T_2. The present experiment revealed that chitosan could be used as an alternative of fungicide to control Phomopsis blight and fruit rot of eggplant. This findings of the study justify with the statements of Benhamou *et al*., (1994) and O'Herlihy *et al*., (2003) that chitosan has been considered as an alternative to chemical fungicides.

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Table 7: Effect of Chitosan and Bavistin on Leaf Blight and Fruit Rot Disease Incidence (DI) of Eggplant in the Field Condition

Treatments	Leaf Blight		Fruit Rot	
	% DI	% Reduction Over T_2	% DI	% Reduction Over T_2
T_1=Untreated Seed (Control-1)	37.5 a	18.18	45.83 b	31.26
T_2=Seed Treated With *P. vexans* (Control-2)	45.83 a	-	66.67 a	-
T_3=Seed Treated With 0.5% Chitosan	4.17 b	90.90	8.33 c	87.51
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	8.33 b	81.82	12.5 c	81.25
T_5=Seed Treated With 0.1% Bavistin	8.33 b	81.82	12.5 c	81.25
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	12.5 b	72.73	16.67 c	74.00

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

Table 8: Effect of Chitosan and Bavistin on Leaf Blight and Fruit Rot Disease Severity (PDI) of Eggplant in the Field Condition

Treatments	Leaf Blight		Fruit Rot	
	PDI	% Reduction Over T_2	PDI	% Reduction Over T_2
T_1=Untreated Seed (Control-1)	35.83 b	23.23	50.00 b	18.92
T_2=Seed Treated With *P. vexans* (Control-2)	46.67 a	-	61.67 a	-
T_3=Seed Treated With 0.5% Chitosan	10.00 d	78.57	8.33 d	86.49
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	15.00 cd	67.86	13.33 cd	78.38
T_5=Seed Treated With 0.1% Bavistin	11.67 cd	74.99	9.17 d	85.13
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	15.83 c	66.08	15.00 c	75.68

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

Fig. 4: Leaf Blight and Fruit Rot of Eggplant Caused by *P. vexans*.

g) Effect of Chitosan and Bavistin on the Yield of Eggplant

Results of the present study indicated that seeds treatment with chitosan and Bavistin 50 WP significantly increased fruit yield over the pathogen treatment (Table 9). The highest (16.51 t/ha) yield was found in the treatment T_3 followed by the treatment T_4 (15.77 t/ha) which were superior to all other treatments but these were statistically identical. The lowest yield (9.03 t/ha) was recorded in the treatment T_2 where eggplant seeds were treated with *P. vexans* spore suspension. Seed treatment with chitosan also increased the size of eggplant fruits. The bigger fruit size (60.27 g) was observed in T_3 which was statistically identical with treatment T_4 (59.83 g). In contrast, smaller fruit size (44.37 g) was found in T_2 followed by untreated control T_1 (50.23 g) and no statistical difference were found among them. Moreover, the maximum number of fruits were recorded in T_3 (164.73) followed by T_4 (158.15) and T_5 (151.33) and no statistical difference were found among them. Chitosan was found to increase 74.64% yield in T_4 over T_2 treatment. These results are approved by Mishra *et al*., 2014 who reported that seed treatment with chitosan increased number of fruits plant^{-1} and yield of tomato

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Table 9: Effect of Chitosan and Bavistin on Yield of Eggplant

Treatment	Total Fruits/Plot	Weight (g)/Fruit	Yield (g/m^2)	Yield (t/ha)	% Increase Over T_2
T_1=Untreated Seed (Control-1)	133.17 d	50.23 bc	1116.58 c	11.17	23.69
T_2=Seed Treated With *P. vexans* (Control-2)	122.17 e	44.37 c	902.97 d	9.03	-
T_3=Seed Treated With 0.5% Chitosan	164.73 a	60.27 a	1651.09 a	16.51	82.83
T_4=Seed Treated With *P. vexans* + 0.5% Chitosan	158.15 ab	59.83 a	1576.61 a	15.77	74.64
T_5=Seed Treated With 0.1% Bavistin	151.33 bc	52.23 b	1318.65 b	13.19	46.06
T_6=Seed Treated With *P. vexans* + 0.1% Bavistin	145.94 c	51.53 b	1251.91 bc	12.52	38.65

* Mean values within the same column having a common letter(s) do not differ significantly (*P=0.05*) by LSD.

IV. Conclusion

From this study it can be concluded that chitosan is a natural substance which biodegradable and non-toxic directly inhibited the growth and reproduction of fungus *P. vexans*. Seed treatment with chitosan induced the defense enzymes, reduced the disease incidence and PDI and increased growth and yield of eggplant. However, supplementary studies are required to confirm the optimal concentration of chitosan to control Phomopsis blight and fruit rot of eggplant caused by *P. vexans*.

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References Références Referencias

1. Abd-El-Kareem, F., EL-Mougy, N. S., EL-Gamal, N. G. & Fatouh, Y. O. (2006). Use of chitin and chitosan against tomato root rot disease under greenhouse conditions. *Research Journal of Agriculture and Biological Sciences. 2*, 147-152.
2. Abraham, J., Anandaraj, M., Ramana, K. V. & Sarma, Y. R. (1996). A simple method for indexing *Phytophthora* and nematode infection in black pepper (*Piper nigrum* L.). *Journal of Spices Aromatic Crops. 5*, 68-71.
3. Algam, S. A. E., Xie, G., Li, B., Yu, S., Su, T. & Larsen, J. (2010). Effects of *Paenibacillus* strains and chitosan on plant growth promotion and control of Ralstonia wilt in tomato. *Journal of Plant Pathology. 92(3)*, 593-600.
4. Ashrafuzzaman, M. H. (1976). Laboratory manual of Plant Pathology, First edition, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. 94 p.
5. Barneet, H. L. & Hunter, B. B. (1980). Illustrated genera of Imperfect fungi. 164 p.
6. BBS. (2017). Yearbook of Agricultural Statistics of Bangladesh. Statistics Division, Bangladesh Bureau of Statistics. Ministry of Planning, Government of the Peoples' Republic of Bangladesh. pp. 252-291.
7. Benhamou, N., Lafontaine, P. J. & Nicole, M. (1994). Seed treatment with chitosan induces systemic resistance to Fusarium crown and root rot in tomato plants. *Phytopathology. 84*, 1432-1444.
8. Bradford, M. M. (1976). Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry. 72*, 248-254.
9. Brine, C. J. Sandford, P. A., Zikakis, J. P. (1992). Advances in chitin and chitosan. Elsever Science Publishers. London. 685 p.
10. Cakmak, I. & Marschner, H. (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant Physiology. 98*, 1222-1227.
11. Chance, B. & Maehly, M. (1955). In: Colowick S. P., Kaplan N. P., eds. Methods in Enzymology. Academic Press. New York. 764 p.
12. Chittenden, C. & Singh, T. (2009). In vitro evaluation of combination of *Trichoderma harzianum* and chitosan for the control of sapstain fungi. *Biological Control. 50*, 262-266.
13. El-Mohamedy, R. S. R., Abdel-Kareem, F., Jabnoun-Khiareddine, H. & Daami-Remadi, M. (2014). Chitosan and *Trichoderma harzianum* as fungicide alternatives for controlling Fusarium crown and root rot of tomato. *Tunisian Journal of Plant Protection. 9*, 31-43.
14. Gomez, K. A. & Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. Second ed. NY, USA: John, Wiley and Sons.

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15. Harada, J. S., Arima, H., Shibayama & Kabashima, R. (1995). Effect of chitosan application on growth and seed yield of soybean. *Marine and Highland Bioscience Center Report. 2*, 15-19.
16. Islam, M. R. & Meah, M. B. (2011). Association of *Phomopsis vexans* with Eggplant (*Solanum melongena*) seeds, seedlings and its management. *The Agriculturists. 9 (1-2)*, 8-17.
17. Jahanara, A., Jannat, R., Hossain, M. M., Ahmed, J. U. & Rubayet, M. T. (2018). Chitosan for plant growth promotion and disease suppression against Anthracnose in chilli. *International Journal of Environment, Agriculture and Biotechnology. 3*, 806-817.
18. Karuna, V., Kumar, S., Vishunabat, K. & Kumar, S. (1994). Location of infection of *Phomopsis vexans* in brinjal seed. *Indian Journal of Mycology and Plant Pathology. 24(3)*, 226.
19. Khan, N. U. (1999). Studies on epidemiology, seed borne nature and management of Phomopsis fruit rot of brinjal. An M. S. Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 42-62.
20. Mahdavi, B. & Rahimi, A. (2013). Seed priming with chitosan improves the germination and growth performance of ajowan (*Carum copticum*) under salt stress. *Eurasian Journal of Bio-Sciences. 7*, 69-76.
21. Majeti, N. A., Kumar, R. (2000). A review of chitin and chitosan applications. *Reactive Functional Polymers. 46(1)*, 1-27.
22. Mandal, S. (2010). Induction of phenolics, lignin and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors. *African Journal of Biotechnology. 9(47)*, 8038-8047.
23. Mian, I. H. (1995). Methods in plant pathology. IPSA-JICA project publication no 24. Institute of Post Graduate Studies in Agriculture. Gazipur. Bangladesh.
24. Mishra, S., Jagadeesh, K. S., Krishnaraj, P. U. & Prem, S. (2014). Biocontrol of tomato leaf curl virus (ToLCV) in tomato with chitosan supplemented formulations of *Pseudomonas* sp. Under field conditions. *Australian Journal of Crop Science. 8(3)*, 347-355.
25. O'Herlihy, E.A., Duffy, E. M. & Cassells, A. C. (2003). The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from plantlets. *Folia Geobotanica. 38*, 201-207.
26. Ortega-Ortiz, H., Benavides-Mendoza, A., Mendoza-Villarreal, R., Ramirez-Rodriguez, H. & Romenus, K. D. A. (2007). Enzymatic activity in tomato fruits as a response to chemical elicitors. *Journal of Mexican Chemical Society. 51(3)*, 141-144.
27. Pan, S. & Acharya, S. (1995). Studies on the seed borne nature of *Phomopsis vexans* (Sacc. and Syd) Harter. *Indian Agriculturist. 39(3)*, 193-198.
28. Rahman, M. M., Ali, M. A., Ahmad, M. U. & Dey, T. K. (2013). Effect of tuber-borne inoculum of *Rhizoctonia solani* on the development of stem canker and black scurf of potato. *Bangladesh Journal of Plant Pathology. 29*, 29-32.
29. Shao, C. X., Hu, J., Song, W. J. & Hu, W. M. (2005). Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling. *Journal of Zhejiang University (Agriculture and Life Science). 31(6)*, 705-708.
30. Singh, R. S. (1992). Diseases of vegetable crops. 2nd Edn. Oxford and IBH publishing company Pvt. Ltd. New Delhi, Bombay, Calcutta. pp. 119-121.
31. Zhao, X. R. & Xia, W. S. (2006). Antimicrobial activities of chitosan and application in food preservation. *Chinese Food Research and development. 27(2)*, 157-160.

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Prevalence of Bovine Fasciolosis and Economic Importance in Wulnchit Municipal Abattoir, Ethiopia

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Abstract- A cross-sectional study was conducted to estimate the prevalence and economic importance of fasciolosis slaughtered at Wulnchit Municipal Abattoir from November, 2011 and April, 2012. A total of 500 livers from cattle selected were examined with systematic random sampling for the presence of liver fluke. Of 500 examined cattle, 171 (34.23%) livers were infected with Fasciola. Both species of Fasciola were identified during the study. These are Fasciola hepatica (F. hepatica) and Fasciola gigantica (F. gigantica). From 171 livers F. hepatica were 120 (70.17%), F. gigantica 30 (17.54%) livers, while mixed infection with both was 11 (6.4 %) animals and 10 (5.8%) cattle were infected with unidentified immature liver flukes. F. hepatica was found to be the predominant fasciola species causing bovine fasciolsis in the study areas. Statistically significant variation was observed in the prevalence of fasciolosis among animals with medium (50%) and good (32.9%) body conditions ($P<0.05$) and animal origin. The economic significance of bovine fasciolosis was also assessed from condemned liver and carcass weight loss. Thus based on the retail value of bovine liver and 1kg of beef the total annual economic loss from fasciolosis during the study time was estimated to be 4, 522,550,000 ETB.

Keywords: *abattoir, bovine, fasciola, prevalence and economic significant.*

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Asefa Getnet [α] & Tegegne Bayih [σ]

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I. Introduction

Livestock in developing countries play crucial role in improving the ever worsening situation between food supply and demand due to human population pressure. Generally animals have a positive impact on diet, Sustainable crop yields, employment prospects and social status of our growing human population (EVA, 2003). Ethiopia posses the largest live stock in Africa with an estimated population of 47.5 million cattle, 26.1 million sheep, 21.7 million goats, 7.8 million equines,1millon camels, and 39.6 million chicken (CSA, 2000), but is not efficiently exploited. The major problem hindering the full exploitation of these resources is animal's disease and traditional management system of domestic animals (Solomon, 1975). The presence of fasciolosis due to *Fasciola hepatica (F. hepatica)* and *Fasciola gigantica (F. gigantica)* in Ethiopia has long been known and its prevalence and economic signify-cance has been reported by several workers (Tadele and Worku, 2006).

Helminth infection on food animal's because significant economic loses (FAO, 1982). They may vary considerably from clinical disease including mortality to chronic production loses which may appear for example as reduced growth rate, weight losses due to reduced animal production, yet, another dimension added by the fact that several helminth infection can be permitted to man (zoonosis). One of the helmithiasis that cause direct and indirect lose, especially in domestic ruminants is fasciolosis. It is serious hazard to efficient production of cattle and sheep (Radiostits *et al*., 2007).

Among the major livestock parasitic diseases responsible for high prevalence and economic losses on livestock production particularly in sheep and cattle is fasciolosis. Bovine fasciolosis is an economically important fasciolidae trimatode of the genus fasciola which migrate in the liver parenchyma and establish and develop in the bile ducts. Fasciolosis mainly affects domestic ruminants, which is caused by the liver fluke parasites. The parasite lives part of its life in aquatic snails and farm animals. The aquatic snails which are found in and around wet areas such as water holes, act as intermediate host. The farm animals act as final hosts which are likely to pick up the parasite if they drink from these sources or eating aquatic plants containing encysted organisms (Okewole *et al*., 2000 and WHO, 1995).

Generally, the distribution of fasciolosis is worldwide, however, the distribution of *F. hepatica,* is highlands of tropical and subtropical regions (Soulsby, 1986). The definitive hosts for *F. hepatica* are most mammals among which sheep and cattle are the most important once. The geographical distribution of trimatode species is dependent on the distribution of suitable species of snails. The genus lymnaea in general and *lymnaea trancatula* in particular is the most common intermediate hosts for *F. hepatica*. In Ethiopia the presence of both *lymnea trancatula (L. tancatula)* and *lymnea natalensis (L. natalensis)* has been reported (Bergeon, 1968 and Garber, 1975). *L. trancatula* is an amphibious snail living in shadow ponds, wet lands, and water troughs while *L. natalensis* is true aquatic mullusk which lives in immersed clear water and slow flowing givers mixed infections by both species of fasciola may

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occur in areas where the ecology is conducive for replication of snail intermediate host. In this species of snail was reported to have worldwide distribution (Urquhart *et al*., 1996).

In Ethiopia, *F. hepatica* is wide spread in areas with attitude above sea level (a.s.l), while *F. gigantic* appears to be the most common species in areas below 1200 meters a.s.l. Both species co-exist in areas with an altitude ranging between 1200-1800 meters a.s.l (Graber, 1975).

Fasciola is fairly large hermaphrodite parasite with a brown leaf shaped body. The anterior end is usually prolonged in to the shape of a cone and the anterior sucker is located at the end of the cone. The ventral sucker is placed at the level of the shoulder of the fluke. Both *F. hepatica* and *F. gigantica* are hamatophagus or blood sucking (Radiostits *et al*., 2007). *F. gigantica* resembles *F. hepatica* but is readily recognized by its large size. The anterior cone in *F. gigantica* is smaller than that of *F. hepatica*, the shoulder are not as prominent as *F. hepatica* and the body is more transparent. Both *F. hepatica* and *F. gigantic* are greyish-brown in colour when they are fresh, but they are changed to gray when preserved. Fasciola eggs have oval shape and yellowish brawn shell with an indistinct operculum (Soulsby, 1982 and Urquhart et al, 1996).

The life cycle of fasciola consists certain phases, adult fasciola live in bile ducts producing eggs, passage of eggs from the host to the outside environment and then subsequent development, hatching of miracidia, then search for and penetration of the intermediate host (snail): *L. trancatula*, development and multiplication of the parasites inside the snail, emergence of the cercariae and then encysted, ingestion of infective metacercaria by final hosts and development to adult worms. Hatching mostly occurs in moist conditions annoyance of the cycle depends on the mortality rate of the snails during the winter, which varies from region to region and from year to year (Radiostits *et al*., 2007).

The pathogenesis of fasciolosis vary according to the stage (phase) of the parasite in liver and the species of the host involved. Essentially the diseases entity can be divided in to acute and chronic form (Soulsby, 1983). Acute fasciolosis is due to massive invasion (migration) of young flukes in the hepatic parenchyma, and the pathological impact is associated to liver damage and haemorrhage. Chronic fasciola infection being the most common form, it is manifested intermes of submandibular oedema, hypoalbuminamia and fasciola eggs in the feces (Soulsby, 1986).

The pathogenic effect usually depends on the number of metacercaria ingested over a period of time and relative suscptibity of the animal. Generally the parasite is capable of developing pathogenic action mechanical by cuticular spines and suckers, predatory by consumption of liver tissue (Urquhart *et al*., 1996).

Both *F. gigantica* and *F. hepatica* can infect human sporadically. Human cases of fasciolosis occur throughout the world. In Ethiopia a case fasciolosis in man has been reported (Taylor *et al*., 2007).In Ethiopia, the prevalence of Bovine Fasciolosis has shown to range from 11.5% to 87% (Taylor *et al*., 2007). *F. hepatica* was shown to be the most important fluke species in Ethiopia. The distribution of *F. gigantica* was mainly localized in the western humid zone of the country that encompasses approximately one fourth of the nation (Tadele and Worku, 2006).

Ethiopian highlands contain pockets of water logged marshy areas which provide suitable habitats year round for the intermediate host of fasciola (Solomon and Abebe, 2007). Though the problem due to fasciola was reported from different parts of the country information on the current status from different location need to be attained, this study aims to fill such gap hence be carried out in cattle in and around Wulnchit.

The objectives of this work are:

- To determine the prevalence of bovine fasciolosis by post mortem examination.
- To identify the commonly involved fluke species.
- To evaluate the direct and the indirect economic losses due to the disease.

II. Materials and Methods

a) Study Area

The study was conducted at Wulnchit municipal abattoir which is located in Wulnchit east shoa zone, 99km south east of Addis Abeba (37.17^{0}N and 8.33^{0}E) with an altitude of 1622 m.a.s.l situated in the well known east African Rift Valley. It has an annual rain fall ranging from 400-800mm and temperature 13.9-27.7^{0}c (National Metrology Service Agency, 1999/2000).

b) Study Animal

The study was conducted on cattle, local breed, originate from neighboring provinces such as; Arsi, Bale, Harar, around Wulnchit area and Borena zones of Ethiopia.

c) Study Design

A cross sectional study was conducted from October, 2011 to April, 2012 by collecting data on events associated with fasciolosis in cattle slaughtered at Wulnchit Municipal Abattoir. After autopsy the liver was inspected grossly, the fluke recovery and count was aimed to be conducted following the approach Hammond and swell, (1990) and identification of the fluke species were carried out by using size parameters described by (soulsby, 1982).

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d) Sample Size Determination

Systematic random sampling method was employed to generate data for the study at the abattoir on cattle presented for slaughter. Thus, taking 73.26% expected prevalence the sample size used for the present study was calculated according to the method described by Thrusfield (1995) as follows:

$n = 1.96^2 pexp\ (1\text{-}pexp)/d^2$

Where; n = required sample size

Pexp = expected prevalence

d = required precision (usually 0.05)

The expected prevalence of fasciola in Wulnchit Abattoir is 73.26% and the confidence interval is 95% with the required precision of 5%. By subtitling the value in the above formula we get sample size (n) =384. But to increase the precision; the sample size is increased to 500.

e) Ant Mortem Examination

During ante-mortem examination detail records about the species, breed, sex, age, and origin and body condition of the animals was recorded. The age estimation was made by using dentitions and owner's information.

f) Post-Mortem Examination

During post mortem examination organs of thoracic cavities specifically liver was systematically inspected for the presence of fasciola by applying the routine meat inspection procedures which consists primary examination followed by secondary examination for the presence of any fasciola. The primary examination involves visualisation and palpation of organ. Whereas secondary examination involves further incisions deep in to the organ incise where a single or more fasciola found.

g) Economic Loss Assessment

The total economic loss due to fasciolosis in cattle slaughtered from the summation of annual liver condemnation cost (direct loss) and cost due to carcass weight reduction (indirect loss) was assessed.

h) Direct Loss due to Organ Condemnation

Direct economic loss was resulted from condemnation of liver affected by fasciolosis. All livers affected with fasciolosis were totally condemned. The annual loss from liver condemnation was assessed by considering the overall annually slaughtered animal in the abattoir and retail market price of an average zebu liver is 50ETB. The information obtained from was subjected to mathematical computation using the formula set by (ogunrinade, 1982).

ALC = CSR X LCX P.

Where ALC = Annual loss from liver condemnation.

CSR = Mean annual cattle slaughtered at Adama municipality abattoir.

LC = Mean cost of one liver in Wulnchit Town.

P=Prevalence rate of the disease at the study abattoir.

i) Indirect Loss due to Carcass Condemnation

A 50% carcass weight loss due to fasciolosis in cattle has been described by Yugoslavian investigator (Polydorous, 1981). Retail market price condemned organs was assessed based on information from local butchers. On the other hand, the indirect economic loss was associated with carcass weight reduction due to fasciolosis. A 10% carcass weight loss due to fasciolosis in cattle was reported by Robertson, (1976). Average carcass weight of an Ethiopian zebu was taken as 126 kg (ILCA, 1992). The annual economic loss because of carcass weight reduction due to bovine fasciolosis was assessed using the formula set by Ogunrinade and Ogunrinade, (1980).

ACW = CSR* CL * BC * P*126 kg.

Where ACW = Annual loss from carcass weight reduction.

CSR = Average number of cattle slaughtered per annum at study abattoir.

CL = Percentage of carcass reduction.

BC = an average price of 1 kg beef in Wulnchit town.

P = Prevalence rate of Fasciolosis at Wulnchit abattoir, 126 kg= Average carcass weight of Ethiopian zebu.

j) Data Management and Statistical Analysis

Data generated from post-mortem meat inspection was recorded and later on entered into Microsoft Excel data sheet and statistical analysis was done. SPSS 20 statistical soft ware was used to analyze the data. Liver condemnation rates defined as proportion of condemned liver to the total number of liver examined. The data obtained during the study was subjected to chi square statistical analysis to see the association between rejection rates of liver, origin, and body condition differences were regarded statistically significant since the p value < 0.05.

III. Results

a) Overall Prevalence

From the total 500 cattle slaughtered during the period from November, 2011 to April, 2012 at *Wulnchit* Municipal Abattoir, 171(34.2%) where found to harbour fasciola from the analysis that was made on the bases of origin and body condition.

b) Species Composition of Fasciola

From a total of 171 infected livers *F. hepatica* was the most commonly encountered parasite with the prevalence of 70.17% while *F. giantica* accounted prevalence rate of 17.54%, mixed infection rate 6.4% and those immature flukes accounted as 5.8% (table 1).

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Table 1: Proportion of Fasciola Species

Species of Fasciola	Number of Livers	Percentage (%)
F. Hepatica	120	70.17
F. Gigantic	30	17.54
Mixed Infection	11	6.4
Immature Fluke	10	5.8
Total	**171**	**100.00**

Table 2: Prevalence of Fasciolosis Based on Origin of the Animal

Origin	No Examined	Positive No (%)	P-Value
Harar	113	5(4.4)	0.006
Wulnchit	92	18(19.6)	0.0012
Arsi	106	68(64.2)	0.006
Bale	112	67(57.8)	
Borena	73	13(17.8)	

Prevalence of fasciolosis based on the animal origin was also assessed and the infection of bovine fasciolosis in animals slaughtered in Wulnchit municipal abattoir was originated from Harar, Bale, Borena, Wulnchit and Arsi. The prevalence is highest (68.2%) in animals brought from Arsi and (57.8%) in animals came from Bale. Both of the above two areas are highlands and this may have high population of snail intermediate host or may have marshy areas. Harar (4.4%), which is midland while Borena(17.8%) and Wulnchit (19.6%) are both low lands resulting lower prevalence of fasciolosis. The origin of animals (table 2) $p=0.006$ which was statistically significant since p-vaue is less than ($P<0.05$).

Table 3: Prevalence of Fasciolosis on the Base of Body Condition

Body Condition Score	No. of Cattle Examined	No. of Positive Cases	No. of Negative Cases	Infection Rate (%)
Medium	38	19	19	50
Good	462	152	310	32.9
Total	**500**	**171**	**329**	**34.2**

Pearson Chi2 (1) =0.035, P-Value = 0.015.

The prevalence of bovine fasciolosis was found to be 50% and 32.9% for medium and Fatty body condition score respectively (Table 3) which was statistically significant ($P<0.05$) indicating body condition was directly related to infestation rate.

c) Economic Loss Analysis

The direct economic loss results from liver condemnation as the result of fasciolosis. The average annual cattle slaughtered was estimated to be 54000, while the mean retail price of bovine liver in Adama town was 50 ETB and the prevalence of fasciolosis in Adama municipal abattoir was estimated to be 34.2%, therefore, the estimated annual loss from organ condemnation is = 9,230,000.00 ETB.

ALC = CSR X LCX P

ALC = 54000×50×34.2=9,230,000.00ETB

The indirect economic loss is due to carcass weight reduction as a result of fasciolosis. From 500 inspected animals 171 were identified as positive in the study abattoir which results in total of 376,110,000ETB monthly losses as a result of carcass weight reduction (indirect loss) during the study. In the study area the average price of 1Kg beef was 60ETB, the annual financial loss from carcass weight reduction due to bovine fascioosis is calculated as follows.

ACW = CSR* CL * BC * P*126 kg

ACW = 54000 × 10% × 60 × 34.2 × 126 = 4,513,320,000ETB

Therefore, the total annual economic loss due to bovine fasciolosis in the study abattoir is the summation of the losses from organ condemnation (direct loss) and carcass weight reduction (indirect loss) with a total of 4,522,550,000ETB equivalent.

IV. Discussions

Both *Fasciola hepatica* and *Fasciola gigantic* have been reported to exist in many parts of Ethiopia. The prevalence of bovine Fasciolosis in Ethiopia varies from 11.5 in buno province (Seyoum, 1987) to 87% in Debre Birhan and abattoir studies have also reported up to 88.57% prevalence of fascioosis in Debre Birhan (Dagne, 1994 and Tsegaye, 1995).

The overall prevalence of bovine fasciolosis in cattle slaughtered at Adama municipal abattoir during

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the study period was 34.2% and this is highly reduced to the earliest prevalence reported in this area 73.26% by Eyakem, (2008). As the result showed the prevalence is closely similar with that of Hagos 33.1 % (2007) at Mekelle Municipal abattoir. The result was lower when compared with higher prevalence reported by Tadele and Worku, (2007) 46.58%, Adem, (1994) in Ziway (56.8%) and Mulualem, (1998) in South Gondar (83.08%), Bahiru and Ephrem, (1979) in Keffa (86%). It is higher when compare with 14.0% at Wolaita Soddo abattoir by Abunna, *et a*l., (2009), Berhe *et al*., (2009) in Mekelle was 24.3%. This variation may be due to the ecological and climatic condition such as altitude, rain fall, and also temperature for the presence of their intermediate snail host. Or due to expansion of veterinary service, awareness created among the people, the advantage of periodically deforming of animals or due to local husbandry condition.

In relation to body condition of the animals, the prevalence was higher in those animals with medium body condition than in those fatty body conditions, 50% and 32.9% respectively. This finding corresponds with the reports of Hagos, (2007). The prevalence reported by this researcher was 33.1, and 29.1% in medium and good body condition animals respectively. This is due to the fact that animals with medium body condition are usually less resistant and are consequently susceptible to infectious diseases.

Species identification revealed that *F. hepatica* was more prevalent (70.17%) as compared to *F. gigantica* (17.57%); certain proportion of animals (6.4%) harboured mixed infestation and others unidentified immature fluke (5.8%). In support of the present study, Dechasa *et al.,* (2012) reported that 45.20% *F. hepatica*, 26.54% *F. gigantica*, 15.72% mixed infections and 12.53% immature flukes. Gebretsadik *et al.,* (2009) reported that 56.42% of cattle were infested with *F. hepatica* and 9.17% with *F. gigantica.* However, in another study (Fufa *et al.,* 2009) stated that the most common liver fluke species affecting cattle at Welaita Sodo was *F. gigantica.* The higher prevalence of *F. hepatica* might be associated with the existence of favourable ecological biotopes for the intermediate host *L. truncatula.*

In relation to origin of the animals the prevalence was higher in those animals brought from Arsi (64.2%) and Bale (57.8%), Harar (4.4%), Borena (17.6%) and Adama (19.6) this indicates that the above two areas have highest value. That may be due to the presence of marshy areas or a large population of the snail intermediate host.

Emphasized on the statement that even if it is realized estimating the actual economic loss due to individual parasitic disease is difficult, this should not be medicate against an attempt to emphasize the cause of the disease. The direct economic loss incurred during this study as a result of condemnation of liver of cattle was estimated about 9,230,000.00ETB per annum and indirect economic loss due to carcass weight reduction was estimated about 4,513,320,000ETB per annum. Therefore, the total annual economic loss due to fasciolsis in the study abattoir is the summation of losses from organ condemnation and carcass weight reduction which is equal to 4,522,550,000ETB. This finding is much higher than the result reported by Tolosa and Tigre, (2007), Adem, (1994) and Daniel, (1995). A total economic loss of about 55,080.00, 154,188 and 215,000 Ethiopian birr per annum in cattle due to fasciolosis at Jimma, Ziway and Dire Dawa municipal slaughter houses, respectively This is probably due to the ecological and climatic difference between these localities.

V. Conclusion

The present study confirmed that fasciolosis is an important disease entity causing considerable loss of revenue due to condemnation of affected liver and carcass weight reduction at study municipality abattoir. This may be due to the fact that the area has suitable ecological condition to the existence and multiplication of the intermediate host (*L. truncatula*).

Based on the aforementioned conclusion, the following recommendations are forwarded:

- Application of good drainage and building of dams at appropriate sites in marshy and low laying areas may reduce the snail problem.
- Locally available control strategies like planting of trees and shrubs that have mollucicidal activity (phytolocia dode candara with local name Endod) along streams should be given special emphasis from economic point of view.
- Keeping the animals off from marshy areas inhabited by intermediate host or by fencing of these risky zones. Creation of awareness to the farmers about the disease shoud be raised to enable them actively participate in the control program.
- Finally, the farmers should be educated and informed about the importance of the disease control programs and regular deworming of animals before and just after rainy season.

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References Références Referencias

1. Abunna, F. Asfaw, A. Megersa, B. and Regass, A. 2009. Bovine Fasciolosis: Coprological, Abattoir Surveyand its Economic Impact due to Liver Condemnation at Soddo Municipal Abattoir, Southern Ethiopia. *Tropical Animal Health and Production*, 42: 289-292.
2. Adem, A., 1994. Prevalence of Bovine and Ovine fasciolosis: A preliminary survey around Ziway Region (Shewa), DVM Thesis, FVM, AAU, Debre zeit, Ethiopia, pp. 14-24.
3. Bahiru, G. and Ephrem, M., 1979. A preliminary survey of bovine fasciolosis in Ethiopia. *journal of Agricultural science* pp 5-12.
4. Berhe, G. Berhane, K. and G. Tadesse, G., 2009. Prevalence and Economic Significance of Fasciolosisin Cattle in Mekelle area of Ethiopia. *Tropical Animal Health and Production*, 41: 1503-1405.
5. CSA, 2000. Central Statistical authority federal democratic republic of Ethiopian Agricultural sample enumeration statistical abstract.
6. Daniel, F., 1995. Economic Importance of organs condemnation due to Fasciolosis and Hydatidosis in cattle and sheep slaughtered at Dire Dawa Abattoir, DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University Debre Zeit, Ethiopia.
7. Dechasa, T. Anteneh, W. and Dechasa. F., 2012. Prevalence, gross pathological lesions and economic losses of bovine fasciolosis at Jimma Municipal abattoir. *Journal of Veterinary Medicine and Animal Health Vol*. 4(1), pp. 6-11, February 2012.
8. Fufa, A. Loma, A. Bekele, M. Alemayehu, R., 2009. Bovinem Fasciolosis: Coprological, Abattoir survey and its economic impact due to liver condemnation at Soddo municipal abattoir, Southern Ethiopia. *Trop. Anim. Health. Prod*., 42(2): 289-292.
9. Gebretsadik, B. Kassahun, B. Gebrehiwot, T., 2009. Prevalence and economic significance of fasciolosis in cattle in Mekelle Area of Ethiopia. *Trop. Anim. Health. prod*., 41(7): 1503-1504.
10. Graber, M., 1978. Helminthes and Helminthiasis of Domestic and wild animal of Ethiopia. *Bulletin of Animal Health and prd.In Africa*, 23: 57-86.
11. Gracey, 1986. Information using dentition to age of cattle. http,//www.fis.usda,go./ofo/fsc/bse/informationhtm.
12. Hagos, A., 2007. Study on prevalence and economic impact of bovine Hydatidosis and Fasciolosis at Mekelle Municipal Abattoir, DVM Thesis, FVM, AAU, Debre zeit, Ethiopia, pp. 15-23.
13. Hammond, J. A. and Sewell, M. M. H., 1990. Diseases caused by helminthes. *In*: M. M. H., Sewell and D. W., Brocklesdy (edirors. Handbook on Animal Diseases in the Tropics, 4th edition. (CTVM Edinburgh University). pp 119-123.
14. ILCA, 1992. International Livestock Center for Africa. Debre Birhan experimental station annual report, p. 46.
15. Mulualem, E., 1998. Epidemiology of Bovine Fasciolosis in weredas of South Gonder administrative Zone bordering Lake Tana. *J. Ethio. Vet. Ass*., 2: 1-14.
16. NMSA, 2006. National Metrology Service Agency, Adama Ethiopia.
17. Nicholson, M. J. and Butterworth, M. H., 1986. A Guide to Condition Scoring of Zebu Cattle. International Livestock Center for Africa- ILCA, Addis Ababa, Ethiopia.
18. Ogunrinade, A. and Adegoke, G. O., 1982. Bovine fasciolosis in Nigeria Intercurrent Parasitic and bacterial infection. *Trop. Ann. H/t.Prod*.14: 121-125.
19. Okewole, E. A. Ogundipe, G. A. T. Adejinmi, J. O. and Olaniyan, A. O., 2000. Clinical evaluation of three chemoprophylactic regimes against ovine helmenthosis in fasciola endemic farm in Ibadan, Nigeria. israel *J. vet. med*., 56(1): 15-28.
20. Radostitis, O. M., Gay, C. C. Hinchlif, K. W. and Constable, P. D., 2007. Veterinary medicine. A text book of cattle, horses, sheep, pigs and goats (10th ed). Sounders Elsevier.
21. Robertson, A., 1976. Hand book on animal disease in tropics, 3: 304.
22. Solomon, W. and Abebe, W., 2007. Effects of strategic antihelmentic treatment intervention bovine fasciolosis: A study conducted in facilities endemic area in north western Ethiopia. *Ethiopia. vet. J.* 11 (2): 59-68.
23. Soulsby, E., 1982. Helminth, Artropods and protozoa of domesticated animals, Bellaire Tindal, London, UK. 7: 40-52.
24. Soulsby, R. L. N., 1986. Helminthes, arthropods and protozoa of domesticated animals. London Bailliere Tindall.
25. Tadele, T. and work, T., 2007. The prevalence and economic significance of fasciolosis at jimma, abattoir, Ethiopia. *Int. J. Vet. med* 2: 1-74.
26. Taylor, M. A., Coop, R. L., and wall, R. L., 2007. Veterinary parasitology 3rd ed. Blackwell C. O. S., Singapore. Tolosa, T. And Tigre, W., 2007. The prevalence and economic significance of bovine fasciolosis at Jimma abattoir, Ethiopia. *Journal of Veterinary Medicine*, 3 (2).
27. Urquhar, G. M., Armour, J. L., Dunn, A. M. and Jennings, F. W., 1996. Veterinary parasitology. 2nd ed. Blackwell science ltd., London, UK.
28. WHO. 1995. Control of food borne Trimatode infections. technical report series 849.

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Annexes

ANNEX 1: Individual Animal Daily Abattoir Recording Format

Ser. No.	ID. No.	Breed	Sex	Species	Body Condition Scores	Fasciolosis	
						+/-	Species

ANNEX 2: Body Condition Scoring

1. Condition scoring 1 (p^-) marked emaciation.
2. Condition scoring 2(p) transverse process project prominently, spines appear sharply.
3. Condition scoring 3(p^+) individual dorsal spines are pointed to the touch hips, tail, head and ribs are prominent.
4. Condition sore 4(m^-) individual dorsa spines clearly visible, muscle mass between hooks and spines are sighty concave.
5. Condition scoring 5(m) ribes usually visible, little fat covers dorsa spines are barely vissibe.
6. Condition scoring 6(m^+) the animal is smooth dorsa spines cannot be seen but are easily felt.
7. Condition scoring 7(G^-) animal is smotth and well coverd but fat deposite are not marked.
8. Condition scoring 8(G) fat cover in critical areas can easily be seen and felt, transvers process cannot be seen or felt.
9. Condition scoring 9(G^+) highly deposited of clearly visible on tail, head, brisket. Dorsal spines, ribs and hooks.

(Source Nicolson and Butterworth, 1986)

ANNEX 3: Age Determination (Estimation) of Cattle from Incisors (Dentition) Teeth

Permanent Incisors	Age
One Pair of Incisors	< 2 Years (Young)
Two Pairs of Incisors	2-3 Years (Young)
Three Pairs of Incisors	3-$3^{1/2}$ Years (Adult)
Four Pairs (Full Set) of Incisors	4-6 Years (Adult)
Medial Incisors Showing Wear and Levelled Tops the Teeth	7-9 Years(Old)
Permanent Incisors Showing Wear and Space Between the Teeth Source: Gracey (1986).	> 10 Years (Old)

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Decomposition of Neem Leaf (*Azadirachta Indica* A.Jus) in Hafir Doka Reserve Forest-Sudan

By Maha Ali Abdelatif

Abstract- Litter Decomposition in forest ecosystems adds nutrients to plants, and represents a significant source of atmospheric CO_2. Despite its essential role in carbon and nutrient cycling, leaf litter decay in reserve forest ecosystems remains poorly studied. A completely randomized block design field experiment was conducted in Hafir Doka forest reserve, (latitudes "ٜ56 "15 015 and" ٜ26 "15 015 N. and longitude 32° 24" 23 "and 32° 13" 23 "E.). The aim of the study is to evaluate organic decomposition of neem leaf and the factors affecting it. One set of 36 litter bags each containing 20 gm. air dried neem leaf were buried 20 cm deep under the canopy of A*cacia tortilis* subsp. *spirocarpa* while another set of 36 bags were used as control, during the period November 2017- February 2018. Random samples of 14 bags were retrieved and taken to laboratory to extract their faunal contents. Soil and air temperature and soil moisture were measured during the sampling events. Decomposers fauna were extracted using Tullgern funnel. Data obtained were statistically analyzed using SPSS design at p= 0.05 and compared according to Pearson correlation coefficient. Results showed that nematodes (Aphasmida), mites (Acari: Oribatida) and Collembola (Insecta, *Entomobryidae*) were extracted as animal decomposers.

Keywords: *neem leaf, decomposition, collembola, mites, nematodes.*

GJSFR-C Classification: *FOR Code: 069999*

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These results suggest that neem leaf litter decay in reserve forests may be affected by plant cover and climatic factors.

Keywords: neem leaf, decomposition, collembola, mites, nematodes.

I. Introduction

Litter decomposition is defined as the process through which organic material is broken down into small particles and mineralized. It occurs through three processes including comminution or fragmentation of detritus, leaching, and catabolism. The rate of decomposition is regulated by prevailing climatic conditions, chemical quality of detritus and decomposer organisms' diversity, (Aerts, 1997, Jones, 1998 & Malhi *et al.*, 2010).

Plant leaf is the main source of adding organic matter and nutrient to the soil compared to the other plant parts. Adding leaf litter to soil improve its physicochemical properties where increased soil moisture trigger the activity of decomposers, (Hossain *et al*,2011, Semwal *et al*, 2003).

The present study is aimed to study decomposition of neem leaf litter and factors affecting it in a reserve forest ecosystem.

II. Material and Methods

a) Study area

Hafir Doka Forest Reserve is located in the semi-dry climate, (latitudes 15^0 15 - 15^0 30 N and Longitudes 32^o 24 - 32 ° - 13 ° E) characterized by the short rainy season with high evaporation and low relative humidity values. Air temperature values show a significant increase in May and fall in July and October due to rainfall. Its soil is a mixture of sandy clay loam and dominated with *Acacia tortilis*.

b) Methods

Neem leaf litter bags were used according to Coleman, *et al*. (2004). 72 bags sized 15 cm X 12 cm X 2 mm diameter. They were filled with 20 gm. air dried neem leaf and then divided into two groups each of 36 bags. One group was buried in a 20 cm deep hole in *Acacia tortilis* Rhizosphere and the second group was used as a control in a completely randomized block design. Decomposition was measured as litter mass t loss obtained by retrieving 14 bags twice a month.

Factors affecting neem leaf decomposition were evaluated in terms of climatic and biological factors. Soil temperature and moisture values were recorded periodically with sampling events. Decomposers fauna in litter were extracted using Tullgern Funnel apparatus, counted and classified to the least possible taxonomic level.

c) Statistical analysis

Statistical Package for Social Sciences (SPSS), was used to analyze and compare data with LSD at 0.05. The Pearson Correlation Coefficient was used to determine the effect of climate factors on the organic decomposition of neem leaf using Statistical Software 8.

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III. Results And Discussion

a) Decomposition of neem leaf litter

The rate monthly neem leaf decomposition was studied and compared between the two study sites. Results obtained shown in Fig. (1), indicated temporal variation of this rate. The rate of decomposition is noticed to be gradually decreasing in the two study sites throughput the study period and generally the decomposition proceeds is greater in the cultivated site than the barren one. The temporal variation of the decomposition could be ascribed to neem leaf composition and decomposability. Similar observation was previously recorded by *Loranger et al, (2002)* who illustrated that as decomposition proceeds; the decomposers usually utilize the soluble and degradable components like sugars, starches and proteins. On the other hand, during later stage, decomposition rate decreases due to the presence of recalcitrant i.e. lignin, cellulose, tannins and hemicelluloses

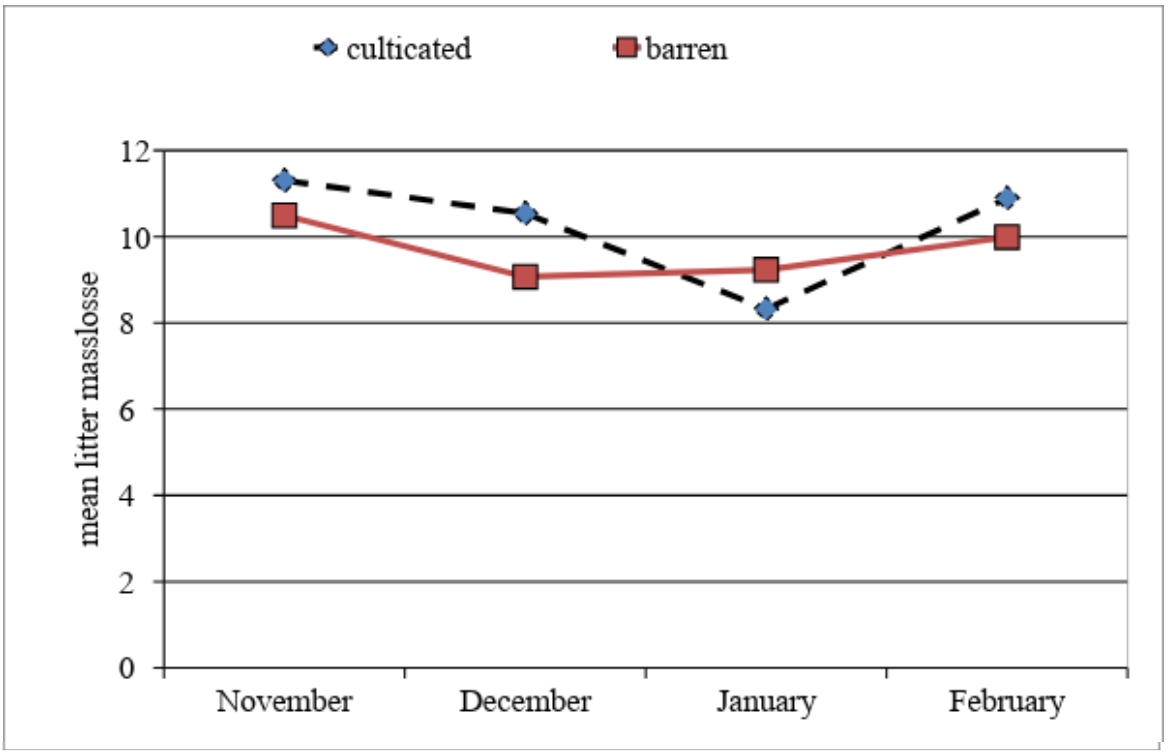

Fig. 1: Temporal variation of neem leaf decomposition in the two study sites

b) Factors affecting neem leaf decomposition

i. *Effect of soil temperature and moisture*

Measurement of temperature and humidity during the period of study shows that the temperature decreased during December and increased gradually in January and February. This pattern of variation applied to the two study sites, (Fig.2). This trend could be attributed to the prevalence of winter season which usually extend over four months started from October and temperature decreases gradually and increased again towards the end of the season.

Soil moisture was measured and compared between the two study sites. Results indicated that the cultivated site is wetter than the barren one except during December as shown in Fig. (3). Asaye, (2017), claimed that Acacia tortilis induced significant impact on soil moisture content.

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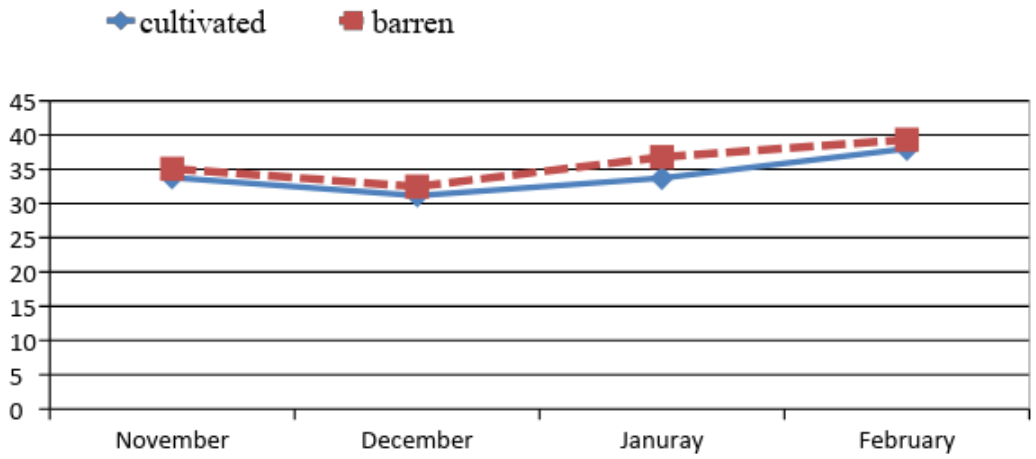

Fig. 2: Variation of soil temperature between the two study sites

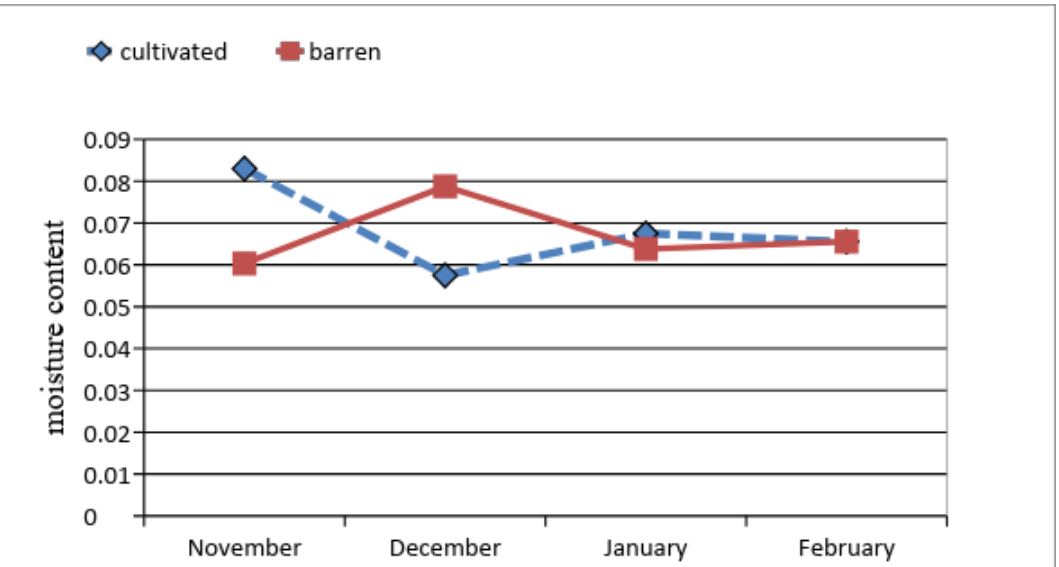

Fig. 3: Variation of soil moisture between the two study sites

Soil temperature and moisture content were correlated to the monthly mean weight loss of neem leaf. Results indicated that temperature was positively correlated to monthly mean weight loss of neem leaf in November in the two study sites, but negatively in the cultivated site in December and January. Monthly mean weight loss of neem leaf was negatively correlated to temperature in the barren site during February.

Monthly mean weight loss of neem leaf correlated to soil moisture content showed positive correlations throughout the study period in the two study sites as given in Table (1). Many studies have quantified the influence of temperature on the rate of litter decomposition and soil respiration. Moore (1986) carried out a laboratory study to relate the decomposition rates of hardwood and coniferous leaf litter with temperature and moisture. He concluded that decomposition rate was found to be a linear function of the temperature and moisture values.

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Table 1: Pearson correlation coefficient for temperature, moisture and monthly mass weight loss of neem leaf litter

Month	Temperature		Moisture	
	cultivated	barren	cultivated	barren
November	0.483846	0.407567	0.083	0.060333
December	-0.48134	0.623474	0.0575	0.078778
January	-0.75574	0.508865	0.0675	0.063625
February	0.371474	-0.12142	0.073	0.065

ii. *Effect of animal decomposers*

Pearson Correlation Coefficient was applied to evaluate the role of animal decomposers on neem leaf decomposition. It was evident that positive correlation was observed between mass loss and animal decomposers individual mean number during November and December and a negative one during January and February except for mite which showed positive correlation during February, (Table 2)._Soil invertebrates are intimately linked to below ground process such as litter decomposition. Their effect depends largely on diversity of organisms and substrate quality. Such dependence showed temporal variations, Endlweberm *et al.*(2006)

Table 2: Pearson correlation coefficient for the effect of animal decomposers on the decomposition of neem leaf litter

Month	Animal Decomposer		
	Nematode	Mite	Collembola
November	0.036752	0.170496	0.352084
December	0.463251	0.36317	0.47343
January	-0.28316	-0.35968	-0.0705
February	-0.04559	0.142001	-0.04994

The effect of nematode on neem leaf litter decomposition was evaluated in term of mean mass loss, in both study sites. Results obtained showed that nematode mean number was correlated to neem leaf mean mass loss by 97.72 % in the cultivated site and 92.38 % in the barren site (Fig. 5 a & b respectively). According to Kimenju et al., (2004), nematodes may accelerate the decomposition of neem leaf and other organic substrate.

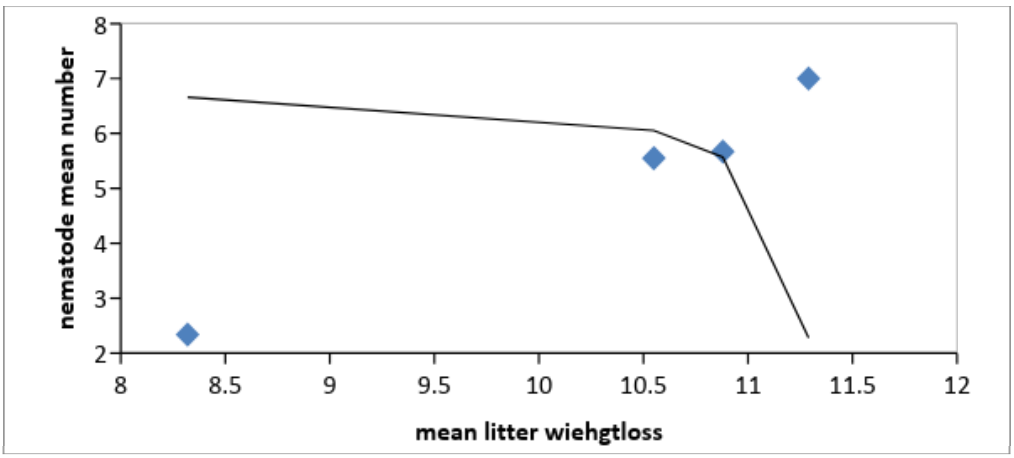

Fig. (4 a): Effect of nematode on decomposition neem leaf in the cultivated site

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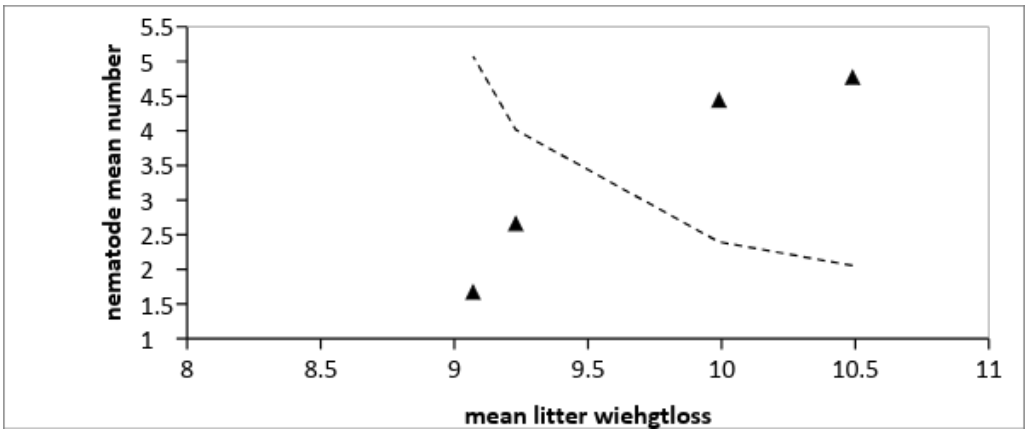

Fig. (4 b): Effect of nematode on decomposition neem leaf in the barren site

The effect of mite on neem leaf litter decomposition was evaluated in term of mean mass loss, in both study sites. Results obtained showed that the mean number of mite was correlated to neem leaf mean mass loss by 69.23 % in the cultivated site and 43. 30% in the barren site (Fig. 6 a & b respectively). Lussenhop, (1980), indicated that mite has significant role in decomposition.

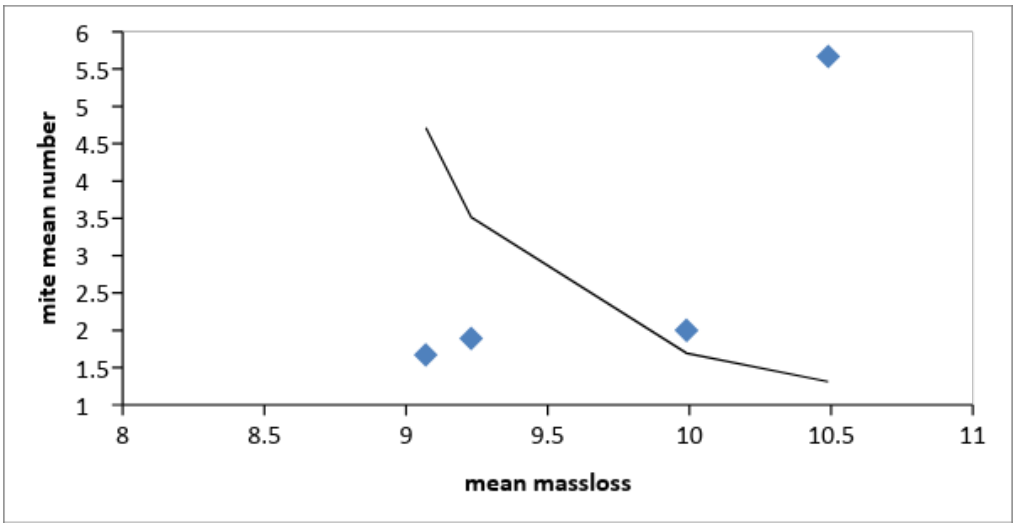

Fig. (5 a): Effect mite on decomposition neem leaf in the cultivated site

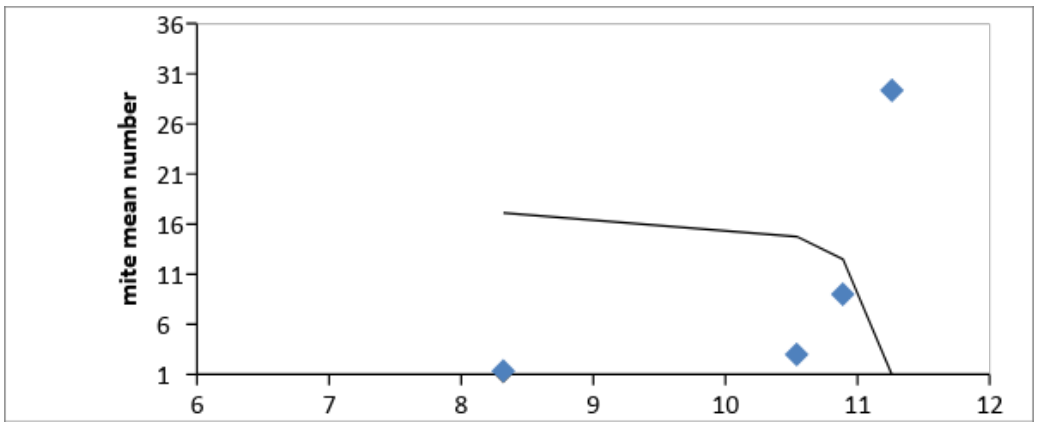

Fig. (5 b): Effect mite on decomposition neem leaf in the barren site

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The effect of Collembola on neem leaf litter decomposition was evaluated in term of mean mass loss, in both study sites. Results obtained showed that collembolan mean number was correlated to neem leaf mean mass loss by 86.60% in the cultivated site and 37.34 % in the barren site (Fig. 7 a & b respectively). Due to their feeding activity; Collembola affect decomposition processes and the microstructure of the soil, Cragg and Bardgett, (2001).

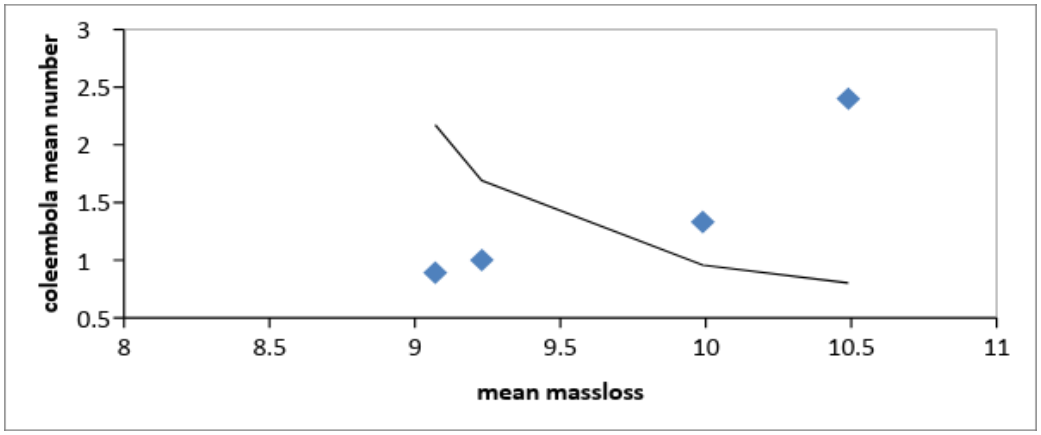

Fig. (6 a): Effect collembola on decomposition neem leaf in the cultivated site

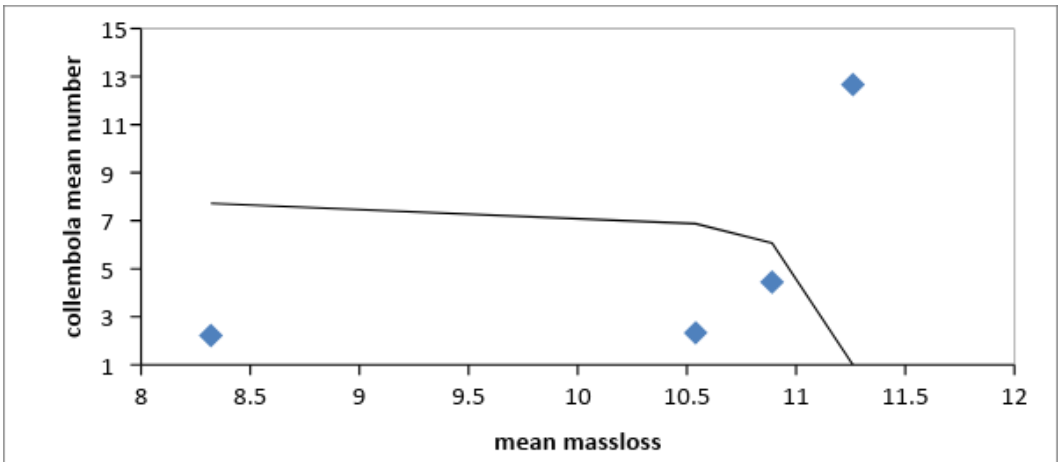

Fig. (6 b): Effect collembola on decomposition neem leaf in the barren site

References Références Referencias

1. Aerts, R. (1997). Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos. 1997; 79:439–449.
2. Asaye, Z.T. (2017). Effects of scattered Acacia tortilis (Forssk) hayne on soil properties in different land uses in Central Rift Valley of Ethiopia. Journal of Sustainable Forestry, 36 (2): 164-176.
3. Cragg, R., Bardgett, R.D., 2001. How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biol. Biochem. 33, 2073–2081.
4. Endlweberm, K. Schadler, M. and Scheu, S. (2006). Effects of foliar and soil insecticide applications on the collembolan community of an early set-aside arable field. Applied Soil Ecology, (31): 136–146.
5. Kimenju, J. , Muirum D., Karanja N., Nyongesa M. and Miano D. (2004). Assessing the role of organic amendments in management of root-knot nematodes on common bean, *Phaseolus vulgaris* L. journal of tropical microbiology and biotechnology, 3: 14-23.
6. Loranger G, Jean-Franccois P, Imbert D, Lavelle P. (2002). Leaf decomposition in two semi-evergreen tropical forests: influence of litter quality. Biology and fertility of soils. 35:247-252.
7. Lussenhop J. (1980): Microbial and micro arthropod – detrital processing in prairie soil. Ecology, (26) 4: 964 –972.
8. Malhi Y. Silman, M. Salinas N, Bush M, Meir P, and Saatchi S. (2010). Introduction: elevation gradients in the tropics: laboratories for ecosystem ecology and global change research. Global change biology, 16:3171–3175.
9. Moore, A.M. (1986). Temperature and moisture dependence of decomposition rates of hardwood and coniferous leaf litter. Soil biology and biochemistry, 18: 427 - 435.

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A New, Phenotypically Distinct Subpopulation of Regulatory Killer T ex-Th17 Cells Expressing $CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}$ $ROR^{low}IL\text{-}17^{low}$

By Maria S. Sayapina
Osaka University

Abstract- Th17 and regulatory T (Treg) cells are integral in maintaining immune homeostasis and Th17– Treg imbalance has been associated with inflammatory immune suppression in cancer. Here it is shown that in addition to ROR+Foxp3+ cells eTreg (Effector Regulatory T Cells) cells are a source of ex-Th17 $CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}$ (Regulatory Killer T – RKT) cells while the latest is much more suppressive. Moreover, we have identified a set of key cytokines that favor the generation and expansion of ex-Th17 $Foxp3^{low}$ cells. These findings should accelerate efforts to define the function of this new subset of Treg cells in the immune response to cancer.

Keywords: *regulatory killer t ex-th17 cells, etregs, $CD4^{low}CD25^{hi}CD49^{hi}foxp3^{hi}ROR^{low}IL\text{-}17^{low}$ cells.*

GJSFR-C Classification: *FOR Code: 069999*

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A New, Phenotypically Distinct Subpopulation of Regulatory Killer T ex-Th17 Cells Expressing $CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}ROR^{low}IL\text{-}17^{low}$

Maria S. Sayapina

Abstract- **Th17 and regulatory T (Treg) cells are integral in maintaining immune homeostasis and Th17– Treg imbalance has been associated with inflammatory immune suppression in cancer. Here it is shown that in addition to ROR+Foxp3+ cells eTreg (Effector Regulatory T Cells) cells are a source of ex-Th17 $CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}$ (Regulatory Killer T – RKT) cells while the latest is much more suppressive. Moreover, we have identified a set of key cytokines that favor the generation and expansion of ex-Th17 $Foxp3^{low}$ cells. These findings should accelerate efforts to define the function of this new subset of Treg cells in the immune response to cancer.**

Keywords: regulatory killer t ex-th17 cells, etregs, $CD4^{low}CD25^{hi}CD49^{hi}foxp3^{hi}ROR^{low}IL\text{-}17^{low}$ cells.

I. Significance Statement

In this work, the new subpopulation of ex-Th17 CD4 lowCD25hi CD49hi Foxp3hi cells has been described. Thus the relative concentration of IL-2, IL-12, IL-1β and IL-23 in the tumor microenvironment may be a critical factor for the generation of exTh17 RKT that will be converted into INF-γ- producing exTh17Foxp3low (exTh17/Th1) cells. Based on these findings, it had been predicted that cytokine milieu (low amounts of TGF-β and high quantity of IL-2, IL-12, IL-1β, and IL-23) in cancer favors the generation and expansion of exTh17Foxp3low cells, although further studies are needed to validate this concept. This knowledge should accelerate efforts to describe the new subpopulation ex-Th17 CD4lowCD25hiCD49hiFoxp3hi (RKT) cells in more detail and create new drugs for several immunogenic types of tumors.

II. Introduction

Treg cells consist of functionally diverse subsets of immune suppressive T cells that play a crucial role in the modulation of immune responses and the reduction of deleterious immune activation [1, 2]. Treg cells may participate in the progression of cancer, especially about the ability of Treg cells to promote the development of tumors [3]. It had been described that the levels of Intratumoral Treg cells correlating with better or worse outcomes depending on the tumor type [4, 5]. Recent studies indicate that human ovarian cancer cell line SKOV-3 could convert, in the presence of IL-2, Treg into Th17 cells. These results support the ability of the tumor microenvironment to regulate and expand IL-17-producing T-helper (Th17) cells. Similar results had been obtained upon stimulation of CD4+ T cells in the absence of tumors but in the presence of IL-1β/IL-6 and IL-2 [6]. Cytokine profile analysis revealed that ovarian tumor cells, tumor-derived fibroblasts, and antigen-presenting cells (APCs) secrete IL-1β/IL-6 [7]. IL-1β is a potent inducer of Th17 cell differentiation and expansion, whereas IL-6 is capable of expanding memory Th17 cells [8]. Gene profile analysis revealed that SMAD 6 and HDAC 11 are hyper expressed in ovarian cancer cell line SKOV-3 [9]. In its turn, Smad6 is transcriptionally induced by the anti-inflammatory cytokine TGF-β. On the one hand, the importance of the concentration of TGF-b had been illustrated in selectively regulating Treg and Th17development. Low concentrations of TGF-b favor Th17 differentiation by enhancing IL- 23 receptor (IL- 23 R) expression, while high amount promote Treg differentiation by inhibiting IL-23 R up-regulation [10]. On the other hand, it had been also described that ovarian tumor cells secreted a high amount of latent TGF-β (inactive form), but the level of active form of TGF-β was very reduced (≤30Pg/ml) or undetectable because of its short half-life [8, 11]. Importantly, most of all types of tumors secrete a high amount of TGF-β. Over expression of HDAC11 has been associated with inhibition of expression of the gene encoding IL-10 and higher IL-12 mRNA expression [12]. IL-12 and IL-23 shared the same IL-12Rβ1 receptor subunit and have been characterized by overlapping effects on target cells. As shown before, IL-23 stimulation is not only crucial for attaining full effect or function but also necessary for double expression of IL-17A and IFN-γ, induction of T-bet and subsequent deviation toward IFN-γproduction[13]. Here, it was provided an insightful mechanism by which $CD4^{low}$ $CD25^{hi}CD49^{hi}Foxp3^{hi}$cells are generated from Treg cells and regulated by cytokines ex-Th17 that favor the generation and expansion $exTh17Foxp3^{low}$ cells.

III. Results

I conducted three experiments aimed at deriving Treg cells using BALB/c mice (1^{st} time) and

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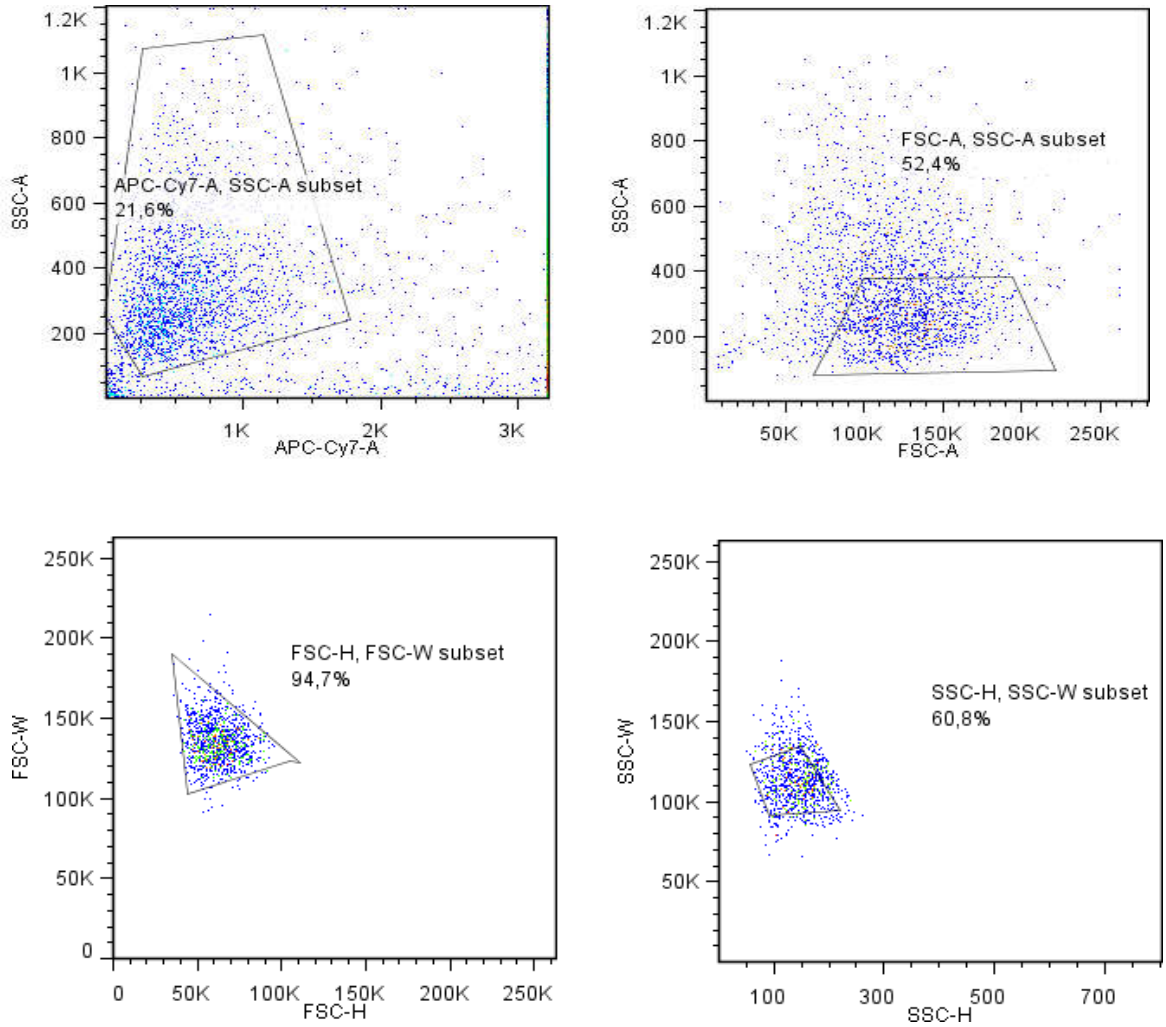
Foxp3-GFP-DTR (2 times), CD4+CD25+FOXP3$^{DTR-GFP}$ cells were isolated from lymph nodes and spleen by flow cytometry cell sorting to high purity and stimulated with anti-CD3/CD28 coated Dyno-beads and IL-2 (Fig. 1). FACS analyses of the isolated population has been at day three after stimulation. As shown in Figure 2, ex-Th17 CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}cells were clearly detectable in populations from the purified CD4+CD25+ T-cell fractions after in vitro expansion. Staining with anti-IL-17 antibody revealed that ex-Th17 CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}cells secreted low level of IL-17, although ROR+FOXP3+ T cells produced elevatedlevel of IL-17 Further experiments revealed that freshly isolated CD4^{low}CD25^{hi}T cells were strongly positive for CD49b and Foxp3 molecules and weakly positive for ROR (Fig. 3).



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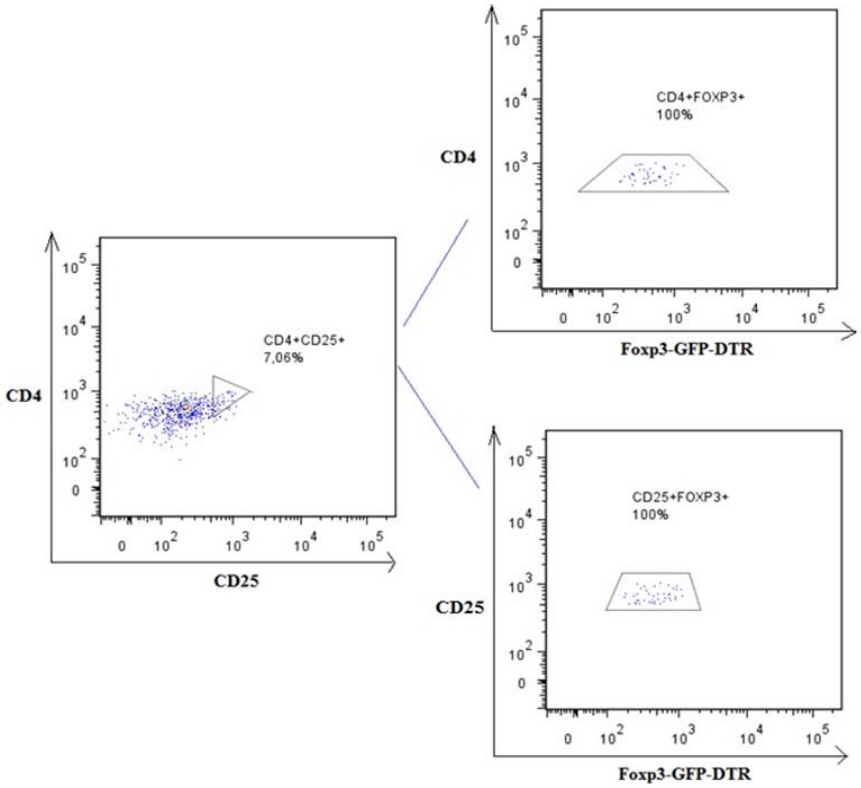
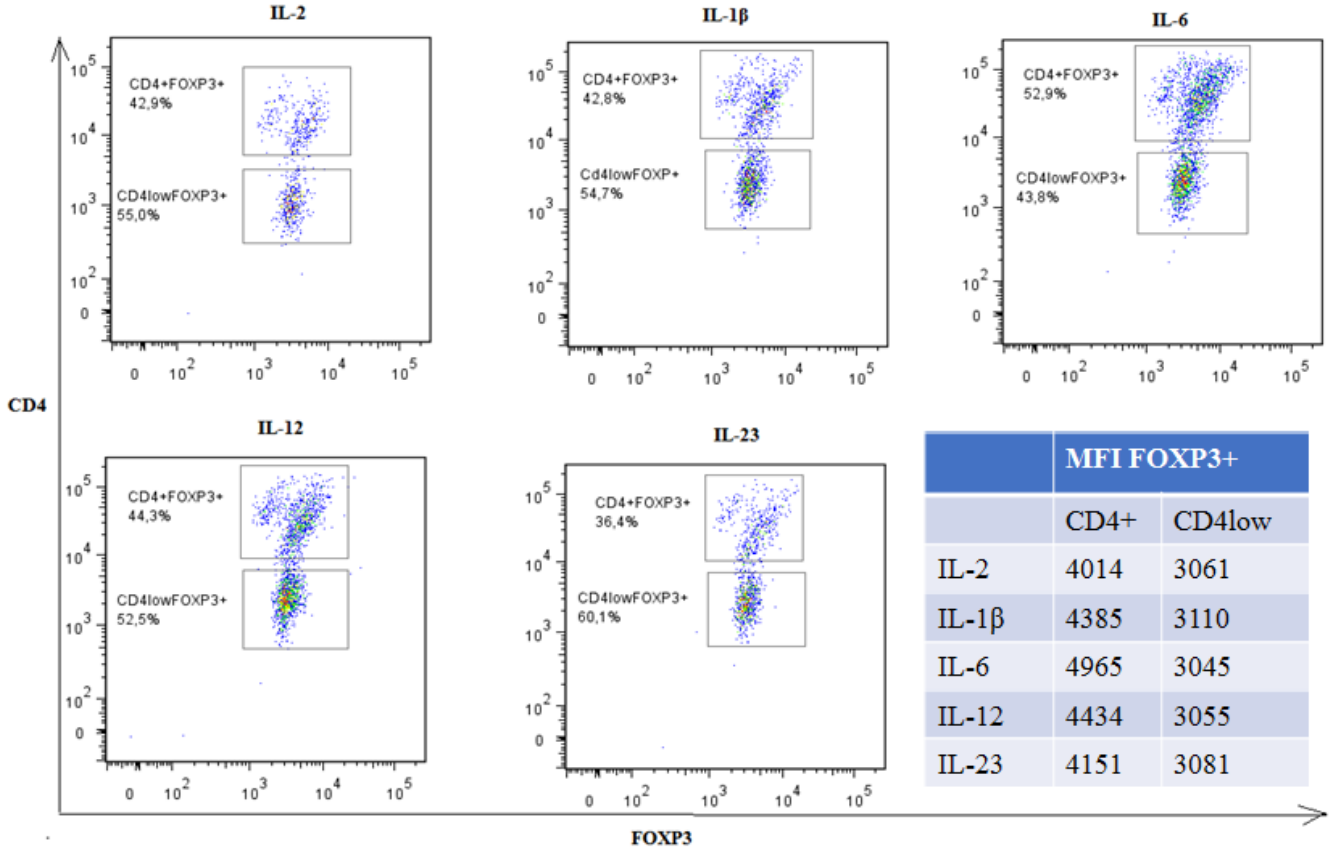


Figure 1: Sorting strategy and the purities of resultant population CD4+CD25+Foxp3+T cells

IL-2 – 100U/ml



	MFI FOXP3+	
	CD4+	CD4low
IL-2	4014	3061
IL-1β	4385	3110
IL-6	4965	3045
IL-12	4434	3055
IL-23	4151	3081

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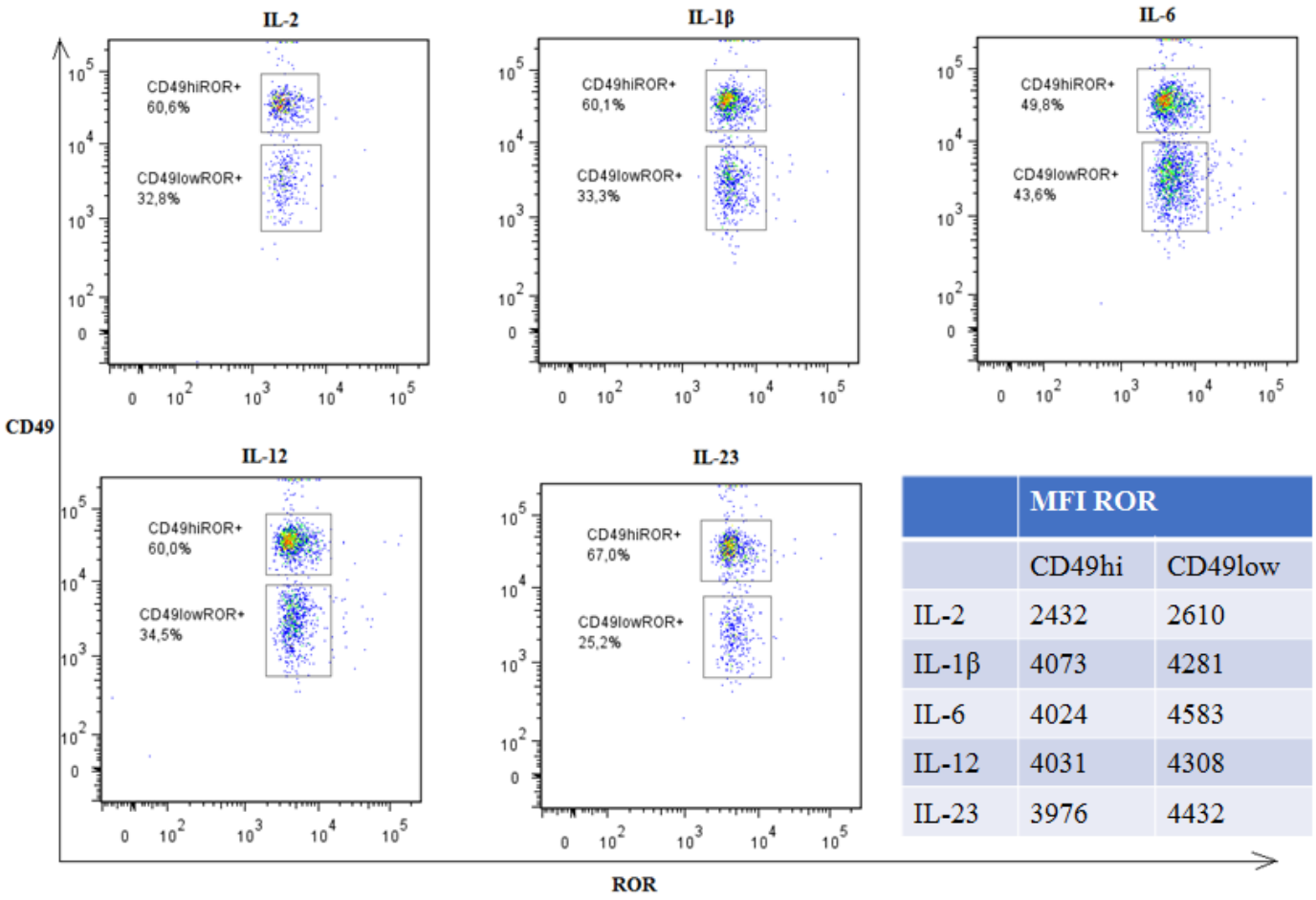
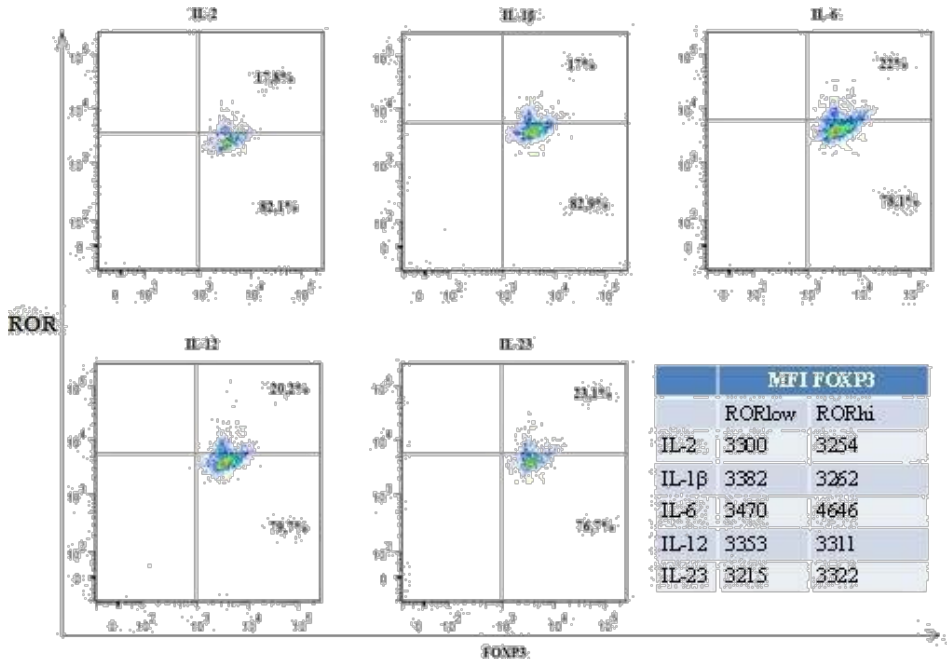


	MFI ROR	
	CD49hi	CD49low
IL-2	2432	2610
IL-1β	4073	4281
IL-6	4024	4583
IL-12	4031	4308
IL-23	3976	4432

Figure 2: CD4^{low}T cells express Foxp3 and CD49b

So I am the first who describe this subpopulation of ex-Th17 CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi} cells.



	MFI FOXP3	
	RORlow	RORhi
IL-2	3300	3254
IL-1β	3382	3262
IL-6	3470	4646
IL-12	3353	3311
IL-23	3215	3322

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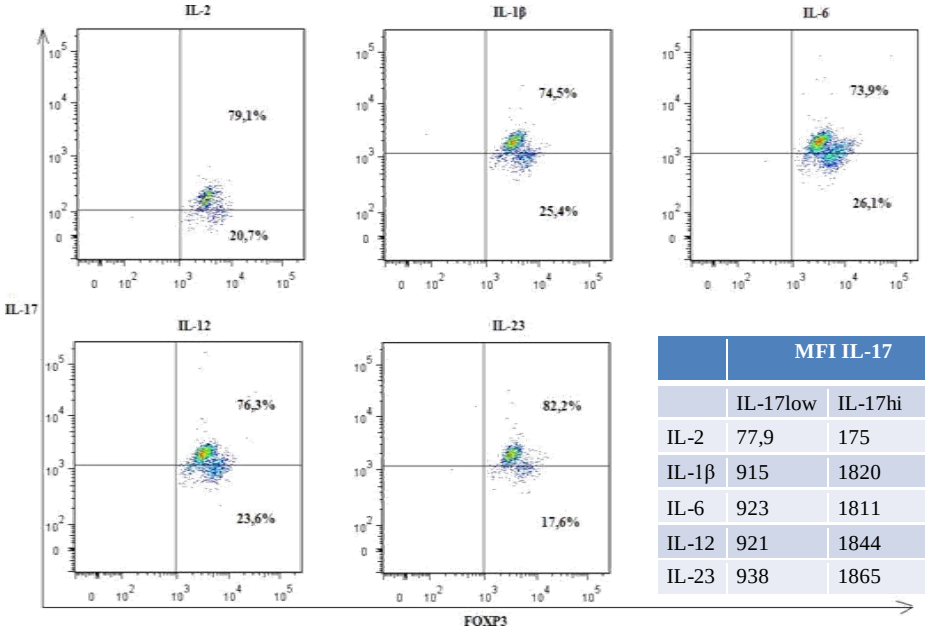
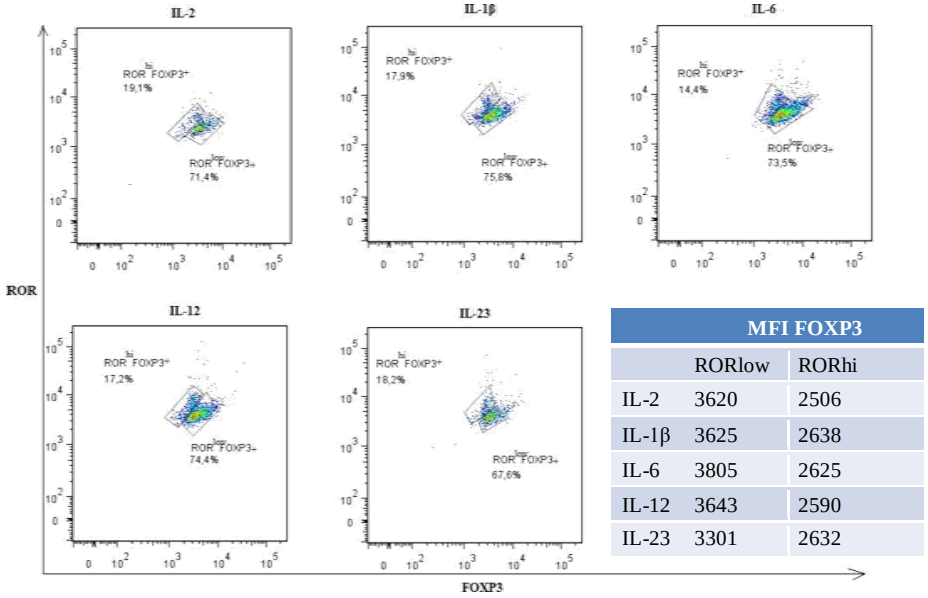


	MFI IL-17	
	IL-17low	IL-17hi
IL-2	77,9	175
IL-1β	915	1820
IL-6	923	1811
IL-12	921	1844
IL-23	938	1865



	MFI FOXP3	
	RORlow	RORhi
IL-2	3620	2506
IL-1β	3625	2638
IL-6	3805	2625
IL-12	3643	2590
IL-23	3301	2632

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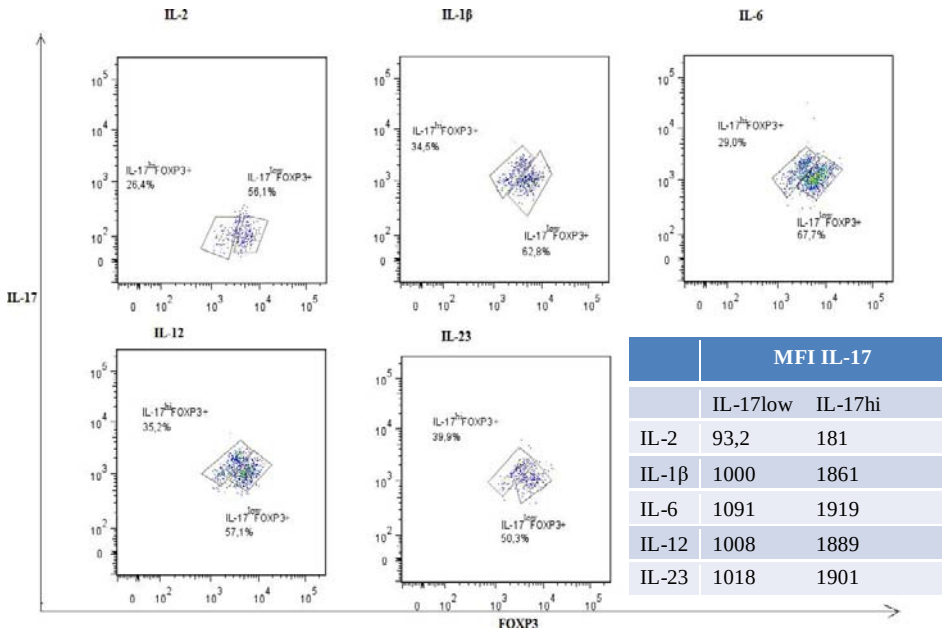

	MFI IL-17	
	IL-17low	IL-17hi
IL-2	93,2	181
IL-1β	1000	1861
IL-6	1091	1919
IL-12	1008	1889
IL-23	1018	1901

Figure 3: CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}cellsexpress low level of IL-17 and ROR

We also decided to determine the role of key cytokines, as discussed earlier, in the generation and expansion of exTh17Foxp3^{low} cells.

In the next stage, we tested the effects of IL-12, IL-1β, IL-6, IL-23 on exTh17Foxp3^{low} differentiation and expansion by using Treg cells from Foxp3$^{DTR-GFP}$ mouse. IL-2-containing medium provided a baseline for comparison. Analysis of ex-Th17 CD4^{low}CD25^{hi}CD49^{hi} Foxp3^{hi} cells revealed that IL-23 plays a more prominent role in the differentiation and expansion of exTh-17Foxp3^{low} cells than do IL-12 and IL-1β. By contrast, IL-6 stimulated IL-17-producing ROR+Foxp3+T suppressive cells.

IV. Discussion

In this work, we have described the new subpopulation of ex-Th17 CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi} cells. Importantly, our data demonstrate the differentiation of eTreg cells to ROR+Foxp3+ cells and exTh17 RKT cells. Bryl et al. have previously reported the population of peripheral blood T cells with reduced CD4 and high CD25 expression (CD4^{low}CD25^{high}), that can non-specifically suppress the proliferation of autologous, previously polyclonally activated CD4+ lymphocytes and to kill them by direct contact. CD4^{low}CD25^{high} T cells expressed significant amounts of both intracellular perforin and granzyme B. At the same time common NK/NKT antigens, including CD16, CD56, CD94, CD158b, CD161 and invariant NKT (iNKT), - were not present on CD4^{low} T cells [14].

Also using whole-genome microarray data sets of the Immunological Genome Project, it was demonstrated a closer transcriptional relationship between NK cells and T cells than between any other leukocytes, distinguished by their shared expression of genes encoding molecules with similar signaling functions, including NT cells and Treg [15]. In terms of common expression of Zap70 and Prscq and potential expression of perforin and granzyme B we concluded that the definition of a CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}cells phenotype is enough to unambiguously detect and study the regulatory function of new subpopulation. It's called Regulatory Killer T – RKT cells which fulfil the current phenotypic criteria identifying the exTh17 RKT cells by simultaneously expressing low amounts of ROR and IL-17A.

Thus, the relative concentration of IL-2, IL-12, IL-1β, and IL-23 in the tumor microenvironment may be a critical factor for the generation of exTh17 RKT that will have been converted into INF-γ-producing exTh17Foxp3^{low} (exTh17/Th1) cells. Based on these findings, it had been predicted that cytokine milieu (low amounts of TGF-β and high quantity of IL-2, IL-12, IL-1β and IL-23) in cancer favors the generation and expansion of exTh17Foxp3^{low} cells, although further studies are needed to validate this concept.

In terms of several types of tumors secrete some cytokines, for example colorectal cancer express high level of IL-23, ovarian cancer – IL-12 (I am planning to prove it) the combination of IL-2, IL-12, IL-1β, and IL-23 in different ways enhanced the differentiation of exTh17Foxp3^{low} (exTh17/Th1) cells from eTreg cells while retaining their ability to expand ROR+Foxp3+ T cells. IL-23 as a critical factor driving exTh17Foxp3^{low} cell expansion. Our findings support the emerging concept that tumor environmental factors drive the generation and expansion of exTh17Foxp3^{low} cells. This knowledge should accelerate efforts to describe the new

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subpopulation ex-Th17 $CD4^{low}CD25^{hi}CD49^{hi}Foxp3^{hi}$ (RKT) cells in more detail and create several drugs for several immunogenic types of tumors (melanoma, ovarian cancer, renal cancer, colorectal cancer) on the basis of IL-2, IL-12, IL-1β and IL-23 that will be delivered locoregionally (intraperitoneally, intrahepatic artery etc) to decrease systemic toxicity.

V. Methods

a) Cell culture

CD4+Tcells that were isolated from FDG mouse by negative selection with mouse CD4+Isolation Kit and were further separated into $CD4+CD25+FOXP3^{DTR\text{-}GFP}$+eTregcells using a FACS ARIA II instrument. Sorted 1,2x10^5 eTreg cells were cultured in the presence of anti-CD3- and anti-CD28-coated (2,5 mcl) Dyna-beads and IL-2 (100U/ml). In some cultures IL-12 (30ng/l), IL-1β (30ng/ml), IL-6 (30ng/ml), IL-23 (30 ng/ml) were added. Cells were analyzed with a FACS Canto instrument 3 days later.

Cells were cultured in culture medium (RPMI-1640 supplemented with 100 U/mL penicillin, 100 g/mL streptomycin, 5 mM 2-mercaptoethanol, 0.05% and 10% fetal bovie serum [FBS]) at 37°C, and 5% CO2, in 96-well round-bottom plates (Greiner, Frickenhausen, Germany).

b) Antibodies and reagents

Allophycocyanin (APC)- and Cy7- conjugated anti-CD4 (RM 4-5) mAb, phycoerythrin (PE) – and Cy7-conjugated ant-CD4(RM 4-5) mAb, peridinin chlorophyll protein complex (PerCP)-andCy5.5-conjugated anti-CD25 (PC-61) mAb, phycoerythrin (PE) anti-ROR (Q31-378) mAbhad been purchased from Bioscience. Brilliant Violet 421(BV421) – conjugated anti-IL-17 (TC-11-18H 10.1) mAb, Alexa Fluor 647-conjugated anti-CD49b (DX5), LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit had been purchased from Biolegend. Recombinant murine IL-6, IL-12, IL-23, IL-1βhad been purchased from Sarstedt and Bioscience.

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The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

All data used to support the findings of this study are included within the article. The research data were performed as part of the employment (Immunology Frontier Research Center, Osaka University).

References Références Referencias

1. Zhang et al. Analysis of CD81 Treg cells in patients with ovarian cancer: a possible mechanism for immune impairment Cellular & Molecular Immunology 2015 12, 580–591.
2. Facciabene A, Motz GT, Coukos G. T-regulatory cells: key players in tumor immune escape and angiogenesis. CancerRes 2012; 72: 2162–2171.
3. Viehl CT, Moore TT, Liyanage UK, Frey DM, Ehlers JP, Eberlein TJ et al. Depletion of CD41CD251 regulatory T cells promotes a tumor specific immune response in pancreas cancer-bearing mice. AnnSurgOncol 2006; 13: 1252–1258.
4. Liu S, Foulkes WD, Leung S, Gao D, Lau S, Kos Z et al. Prognostic significance of FOXP31 tumor infiltrating lymphocytes in breast cancer depends on estrogen receptor and human epidermal growth factor receptor-2 expression status and concurrent cytotoxic T-cell infiltration. BreastCancerRes 2014; 16: 432.
5. Salama P, Phillips M, Grieu F, Morris M, Zeps N, Joseph D et al. Tumor-infiltrating FOXP31 T regulatory cells show strong prognostic significance in colorectal cancer. J ClinOncol 2009; 27: 186–192.
6. Leveque et al. Interleukin 2-mediated Conversion of Ovarian Cancer-associated CD4+ Regulatory T Cells Into Pro in flammatory Interleukin 17-producing Helper T Cells. J Immunother2009; 32: 101–108.
7. Nilsson MB, Langley RR, Fidler IJ. Interleukin-6, secreted by human ovarian carcinoma cells, is a potent proangiogenic cytokine. CancerRes. 2005; 65: 10794–10800.
8. Miyahara et al. Generation and regulation of human CD4+IL-17-producing T cells in ovarian cancer. PNAS; October 7, 2008; vol. 105, no. 40; 15505–15510.
9. http://amp.pharm.mssm.edu/Harmonizome/gene_set/SKOV3/CCLE+Cell+Line+Gene+Expression+Profiles.
10. Zhou et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing ROR gammat function. Nature.2008 May 8; 453(7192): 236-40.
11. Carr TM, Adair SJ, Fink MJ, Hogan KT (2008) Immunological profiling of a panel of human ovarian cancer cell lines. CancerImmunolImmunother 57: 31–42.
12. Villagra et al. The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. NATURE IMMUNOLOGY. VOLUME 10 NUMBER 1 JANUARY 2009; pp. 92-100.
13. Hirota et al. Fate mapping of IL-17-producing T cells in inflammatory responses. nature immunology VOLUME 12 NUMBER 3 MARCH 2011 pp. 255-260.
14. Bryl et al. Human CD4low CD25high regulatory T cells indiscriminately kill auto logous activated T cells. Immunology 2009, 128, e287–e295.
15. Bezman et al. Molecular definition of the identity and activation of natural killer cells. NatureImmunology VOLUME 13 NUMBER 10 OCTOBER 2012 1000-1009.

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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.

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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.

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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.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
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
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
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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