

^{1,2}Alemyar S., ^{1*}Habibi A., ¹Akimbekov N.Sh.

¹Department of Biotechnology, Biology and biotechnology faculty of al-Farabi Kazakh National University, Kazakhstan, Almaty

²Department of Plant Science, Agriculture faculty of Alberoni University, Afghanistan, Kapisa

*e-mail: ainullah.habibi1@yahoo.com

EFFECT OF HYSSOP EXTRACT AGAINST MICROORGANISMS ISOLATED FROM WHEAT GRAINS

The hyssop essential oils have been widely used as bactericidal, fungicidal, antiviral and insecticidal agents. On the other hand, the main method to control bacterial contamination is using synthetic bactericide, but the development or conjugation of bacteria resistance to chemical products, the high operational cost and environmental pollution have created the need for developing alternative approaches to control many microbial contamination, especially bacterial and in this sense the essential oils of hyssop is an alternative to control many pathogenic bacteria.

Because of a growing concern about relationships between diet and diseases and a growing need to improve the quality and safety of food supply, research is to define conditions that minimize the levels of microbial effect compounds wheat germination and growth. Essential oil of hyssop (*Hyssopus officinalis*) revealed one of the best biological compounds which control the pathogenic fungal, viral, bacterial and insect contamination. The results of this experiment displayed essential oils were fully effectiveness on bacterial inoculant and support the growth of wheat grain. It capable to eliminated the all pathogenic bacteria which inoculated with seeds.

The experiment displayed that bacterial inoculant effected on seed germination, only two varieties of wheat capable to geminate with *Pseudomonas* inoculated that was 40% in Ghory cultivar and 80% in Sulh variety. Thus shown bacterial inoculated able to decrease 60 and 20 percentage germination of the Ghory and Sulh varieties. Although in all other wheat cultivars hadn't germination in bacterial inoculated variant, but in controls (inoculant of water, inoculated with essential oil of hyssop) had 100% germination and also in treated variants had > 80% germinated and were greatly treated by essential oil of hyssop (*Hyssopus officinalis*).

Key word: essential oils, environmental pollution, bacterial inoculant, wheat grain, hyssop.

^{1,2}Алемьяр С., ^{1*}Хабиби А., ¹Акимбеков Н.Ш.

¹Биотехнология кафедрасы, биология және битехнология факультеті, әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.

²Өсімдік шаруашылығы кафедрасы, ауыл шаруашылығы факультеті, әл-Берони университеті, Ауғанстан, Капис қ.

*e-mail: ainullah.habibi1@yahoo.com

Бидай дәндерінен бөлініп алынған микроорганизмдерге иссоп сығындысының әсерін зерттеу

Иссоптың эфир майлары бактерицидтік, фунгицидтік, антивирустық және инсектицидтік агент ретінде кеңінен қолданылады. Негізінен, әртүрлі бактериялық аурулармен күресудің басты әдісі ретінде синтетикалық бактерицидтер қолданылады, алайда бактериялардың химиялық препараттарға төзімділігінің артуы, жоғары эксплуатациялық шығын және қоршаған ортаны ластауы микробты зиянкестердің өсуін бақылайтын қосымша жаңа үдерісті жасау керектігін қажет етеді, осы орайда иссоптың эфир майлары ауылшаруашылық дақылдарын қорғауда нағыз қажетті әдістің бірі ретінде қарастыруға болады. Сонымен қатар, токсинді заттар мен зиянды микроорганизмдермен ластанған тамақтанудың рационы қазіргі таңда өте жоғары

алаңдаушылық тудырып отыр. Әсіресе, патогенді және шартты-патогенді микроорганизмдердің көбеюі әртүрлі аурулардың пайда болуына алып келді, олар әдетте сақтау барысында бидай дәні мен бидай өнімдерінің жылдам бүлінуіне соғады. Иссоп (*Hyssopus officinalis*) эфир майының құрамында саңырауқұлақтар, вирустар, бактериялар мен споралардың өсуін шектейтін ерекше биологиялық қосылыстар бар. Жұмыстың нәтижелері эфир майының микроорганизмдердің өсуін толық тежейтін және бидай тұқымының өсуіне ешқандай кері әсер бермейтіндігін көрсетті. Тәжірибеде бидай тұқымдарының өнуі мен өсуін 5 күн барысында бақыланды.

Алынған нәтижелер бидайдың басқа сорттарымен салыстырғанда тек гури мен сулх сорттары бактериялық инокулят *Pseudomonas* sp. қатысында өсуге қабілетті екендігін көрсетті, тұқымдардың өнуі сәйкесінше 40% және 80% құрады. Олай болса, *Pseudomonas* sp. бактериясы клеткаларымен инокуляцияланған өсімдіктердің тұқымдары өте төмен өсу көрсеткіштеріне ие болды. Егуге дейінгі тұқымдарды иссоптың эфир майларымен өңдеу оның өнуі мен тамыр жүйесінің өсуін >80% белсендірді, әрі марфометриялық көрсеткіштерін өзгерісіне алып келді.

Түйін сөздер: эфир майлары, қоршаған ортаның ластауы, бактериялық инокулят, бидай тұқымы, иссоп.

^{1,2}Алемьяр С., ^{1*}Хабиби А., ¹Акимбеков Н.Ш.

¹Кафедра биотехнологии, факультета биологии и биотехнологии
Казахского национального университета имени аль-Фараби, Казахстан, г. Алматы

²Кафедра растениеводства, факультет сельского хозяйства
Университета аль-Берони, Афганистан, г. Каписа

*e-mail: ainullah.habibi1@yahoo.com

Изучение влияния экстракта иссопа на микроорганизмы, выделенные из зерна пшеницы

Эфирные масла иссопа широко применяются в качестве бактерицидных, фунгицидных, противовирусных и инсектицидных агентов. С другой стороны, основным методом борьбы с бактериальными заболеваниями является синтетический бактерицид, но формирование и развитие устойчивости бактерий к химическим препаратам, высокие эксплуатационные затраты и загрязнение окружающей среды повышают потребность в разработке альтернативных подходов к процессу контроля микробной обсемененности, и в этом отношении эфирные масла иссопа являются дополнительным способом защиты сельскохозяйственных культур.

Все большую озабоченность вызывают нездоровый рацион питания, загрязненные токсическими веществами и вредными микроорганизмами. Последнее крайне актуально, поскольку с ростом патогенных и условно-патогенных бактерий значительно участились случаи различных заболеваний, а их обсемененность вызывает быструю порчу зерна и продуктов при хранении. В составе эфирного масла иссопа (*Hyssopus officinalis*) обнаружено одно из лучших биологических соединений, которое контролирует рост патогенных грибов, вирусов, бактерий и спор. Результаты этой работы показали, что эфирные масла полностью подавляют рост микроорганизмов, при этом не оказывают никакого отрицательного воздействия на зерна пророщенной пшеницы. В ходе эксперимента проводились наблюдения за прорастанием семян в течение пяти дней.

Полученные данные показали, что только два сорта пшеницы Гури и Сулх способны прорасти при условии наличия бактериального инокулята *Pseudomonas* sp., развитие семян при этом составляет соответственно 40% и 80%. Таким образом, показано, что растения, инокулированные клетками бактерии *Pseudomonas* sp., имеют низкий темп роста. Предпосевная обработка семян эфирным маслом иссопа (*Hyssopus officinalis*) привела к повышению активности прорастания, роста и развития корневой системы на 80% и изменение морфометрических показателей.

Ключевые слова: эфирные масла, загрязнение окружающей среды, бактериальный инокулят, пшеничное зерно, иссоп.

Wheat (*Triticum aestivum* L.) has taken place as a staple food source for humanities since times immemorial (Satish S., *et al.*, 2010: 237-241). It is considered as a major agricultural commodity and dietary component across the world and it is one of the most significant cereals in view of nutritional value (Abd El-Baky H., *et al.*, 2009:

77-83). Pathogenic microbes cause root infection (rots), wilts and damping off diseases which are a huge problem in agricultural production worldwide. Intensive production with successive cultivations of susceptible crops often results in the arise of high population densities of pathogenic microbes Soil-borne pathogens cause economically important

diseases in planted crops such as damping-off, root rots, seedling blights, crown rots, foot rots, root browning and wilts. The diseases may be caused by more than 50 different fungal species, various bacterial species and viruses. The damage evoked by soil borne pathogens is usually under estimated, since it appears below ground. Plant diseases caused by pathogens below ground may be considered as limiting factors of plant health and save yield quantities and qualitatively. Function of fungicides and bactericide to control soil-borne fungal and bacterial pathogens, with the exception of seed treatments, seems not to be appropriate for economic and ecological reasons (Abawi G. and TL Widmer 2000: 37-47, Bonanomi et al., 2006: 571-578, Macias et al., 2007: 327-348). Efficiency of field crops should be increased to meet the rising global demand for agricultural products. In agricultural farms, pathogenic bacteria are responsible for a sizable part of the economic loss by means of reducing the quality and quantity of the products (Rassaeifar M., *et al.*, 2013: 73-81).

Infested wheat grains are the carry pathogens which can have long-distance diffusion. The major impact of seed-borne diseases in wheat is not only yield reduction but also deteriorate of marketable grain quality. Early detection of pathogens is a crucial step in the diagnosis as well as for the management programs in wheat (Majumder D., *et al.*, 2013: 500-507). Seeds commonly contain high microbial loads, ranging between 10^3 and 10^6 CFU/g which are constituted mainly of pseudomonads, enterobacteria, lactic acid bacteria and yeast (Prokopowich D. and G. Blank 1991: 560-562, Randazzo, C., *et al.*, 2009: 484-490 & Robertson, L.J., *et al.*, 2002: 119-126). These levels can increase during sprouting, reaching populations as high as 10^8 – 10^{11} CFU/g (Ghandi, M. and K.R. Matthews 2003: 301-306, Penas, E., *et al.*, 2008: 698-705 & Peles F., *et al.*, 2012:53-60). Therefore, to minimize microbial contamination of seedlings prior to consumption, initial microbial load should be controlled (Akbas M. Y. and Olmez H. 2007: 619-624).

Naturally happening wheat grains and their products toxicants can antagonistically affect the nutritional quality and safety of foods. Because of a growing concern about relationships between diet and diseases and because of a growing need to improve the quality and safety of our food supply, research is needed to define conditions that minimize the levels of microbial effect compounds wheat germination and growth. Thus, in order to improve wheat grains safety, there is a need for technologies to inactivate or inhibit pathogenic

microbes with essential oils of hyssop that is neutral on environment and compatible natural compounds and plant extracts.

Essential oils can act as prooxidants affecting inner cell membranes and organelles such as mitochondria (Betts 2001: 33-46, Pichersky et al., 2006: 808-811). Depending on type and concentration, they exhibit cytotoxic effects on living cells but are usually nongenotoxic. Because of the great number of constituents, essential oils seem to have no specific cellular targets. As typical lipophiles, they pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. Cytotoxicity appears to include such membrane damage. In bacteria, the permeabilization of the membranes is associated with loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool (Knobloch et al., 1989: 119-128, Sikkema et al., 1994: 8022-8028, Helander et al., 1998: 3590-3595, Ultee et al., 2002: 1561-1568, Di Pasqua et al., 2006: 2745-2749, Turina et al., 2006:101-113) Essential oils can coagulate the cytoplasm and damage lipids and proteins. Damage to the cell wall and membrane can lead to the leakage of macromolecules and to lysis the pathogenic microorganisms (Bakkali F., *et al.*, 2007: 446-475).

Methods and materials

Experimental site and extraction of the essential oils

The study was performed in the laboratory of Microbiology at Department of Biotechnology, Faculty of Biology and biotechnology al-Farabi Kazakh National University. To prepare essential oils, seeds of hyssop were collected from Panjshir province of Afghanistan after the harvesting process and storage at room temperature for 5 days (Shokouhian A., *et al.*, 2016:13-17).-General methods such as steam distillation or isolation with water without any solvents are used in industrial sectors obtain of hyssop essential oils (Jirasek V., *et al.*, 1986: 15-16). To study the effectiveness of vary solvents and their effect on the quantity and composition of essential oil of hyssop was isolated by petroleum ether, ethylene oxide and dichloromethane. The time period of extraction was from 1 to 90 minutes at temperatures of (in petroleum ether 50°C, in ethylene oxide –5°C and in dichloromethane 20°C. The best result was received with petroleum ether at 50°C for 90 minutes. The

method was more effective than other methods, the essential oils production of hyssop raised from 0,92% extract ethylene oxide and dichloromethane to 1,49% extracted by petroleum ether.

The isolated oily liquid 0,15–0,76% pigments are light yellow in color, of pleasant spicy, herb-like, slightly camphoraceous smell with $\rho = 0,917\text{--}0,965$; $\alpha = -6^\circ$ to -25° ; acid number ≤ 4 . According to some scholarly sources the hyssop (*Hyssopus officinalis*) contains more than 1% of essential oil with maximum content at the onset the flowering stage. The content of essential oils (0,90–0,92%) in plants of *Hyssopus officinalis* var. *seravschanicus* demonstrated grown in the area Sukhandarya in Russia is almost in agreement with the latter reference. Leaves had the highest content of essential oil at the stage of budding formation (Kizil S., et al., 2008: 273-279, Diaconu P. and Birza T. 1991: 13-16). Newer papers report higher contents – up to 7% – in relation to genotype as displayed by analyses of the hyssop (*Hyssopus officinalis*) population grown in 1985–1990. The essential oil enable to find in 6 phenotype representatives (corolla color from dark blue to white) was conditioned by flower color (higher content was found in darker-color individuals). Shoots without leaves and non-green plant parts did not contain any essential oil (Jankovsky M. and T. Landa 2012:119-123).

Seed treatment and collection of the data

Petri-plates containing nutrient agar medium were double streak technique and put into the incubator for 24 hour and plates were incubated at 37 °C. Already by using pour plate and spread plate techniques isolated the bacterial contains (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*) of all wheat seeds varieties (Mukawm, Chunta, Ghory, Sosan, Sulh 2002, Akozet, Farblang and Parwa) and save into the test tubes in cool temperature inside the freezer. After re-cultivated of bacteria by adding 10ml of solvent 0.5% NaCl enable to pour from petri to test tubes and 10 seed of wheat in each of specific tubes again put to the incubator for 2 hour in 37 °C. Thus we had four variant Control, bacterial inoculant, treated and essential oils. Ten wheat seeds were kept in each Petri plate containing two disks of Whatman No.1 filter and the experiment was conducted in duplicate (Perello A., et al., 2013: 318-332). Observation on seed germination was taken for five days. The experiment was conducted at room temperature of 25 + 1 °C and 12 hour light and the same darkness. The germinated seeds were counted by emerging radicle

elongating to 2 cm during 5 days of germination. The number of germinated seeds in each Petri dish was daily recorded. The germination percentage was estimated using the following simple equation:

$$Ge = \frac{\text{The number of germinated after five days}}{\text{Total number of seeds}} \times 100$$

Indeed, the magnitude of the Ge index displays a too degree of germination for treated seeds representing high effect of essential oils on Ge. (Rani B., et al., 2014: 1055-1063).

Results and discussion

The study was referred to four variant that each of them had vary of each other effective on wheat germination and growth. The best biological compound which capable successfully controls the pathogenic fungal, viral, bacterial and insect contaminations is essential oils of hyssop (*Hyssopus officinalis*). The results of this experiment revealed essential oils were completely effects on bacterial inoculant and support the growth of wheat grain. It enables to eliminate the all pathogenic bacteria which inoculated with seeds.

The experiment displayed that bacterial inoculant effected on seed germination, only two varieties of wheat capable to geminate with *Pseudomonas* inoculated that was 40% germinated seed in Ghory cultivar and 80% germinated seed in Sulh variety. Thus shown bacterial inoculated caused to decrease 60 and 20 percentage germination of the Ghory and Sulh varieties respectively. Although in all other wheat cultivars hadn't germination in bacterial inoculated variant, but in controls (inoculant of water, inoculated with essential oil of hyssop) had 100% germination and also in Treated variants the wheat seeds germinated 80% and were greatly treated by essential oils of hyssop (*Hyssopus officinalis*) (Figures 1 and 2).

Thus clarified inoculated microbes enable to reduced and eliminated the wheat germination and very significant to inoculant with essential oils of hyssop for combine and prevent this peril before planting the wheat seed.

Mostly of these wheat grains after germination had died and couldn't capable to survive and growth it replayed the colonization of bacteria that appeared black spots around the hypocotyls and epicotyls of wheat seed which caused interfering by pathogenic bacteria that inoculated already with wheat grains and amount of hazard in wheat field. Also effected on

speed of growth which causes to delay the maturity and harvesting process the growth conducted after emerging radicles five days. Generally the wheat germination of controls start at minimum 2 cm and

prolong during five days the highest length shoot of controls variants among all of variants is Sulh variety that had 17 cm in essential oil and 15.7cm in distillate water (Figure 3).

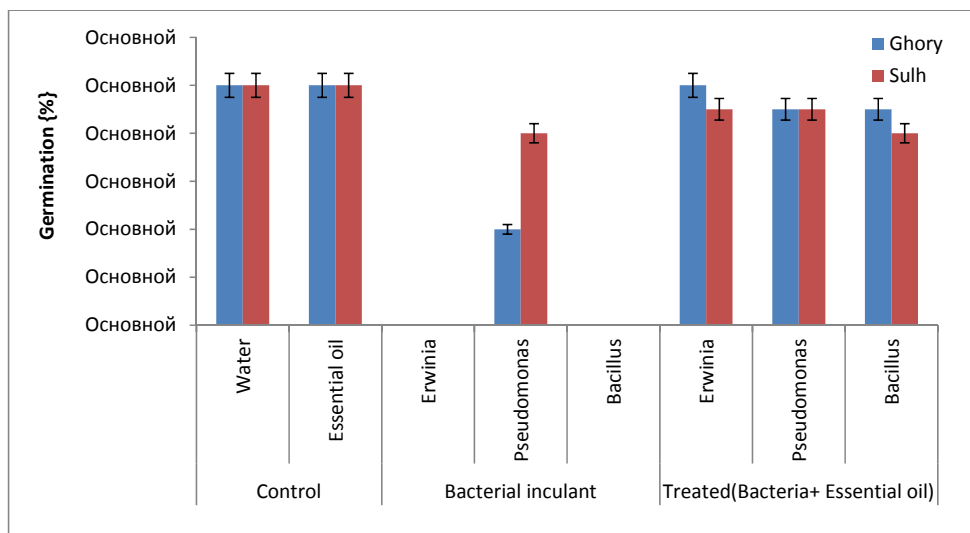


Figure 1 – Percentage of two varieties of wheat grains germination in controls and different variants contaminated with *Erwinia*, *Pseudomonas* and *Bacillus*

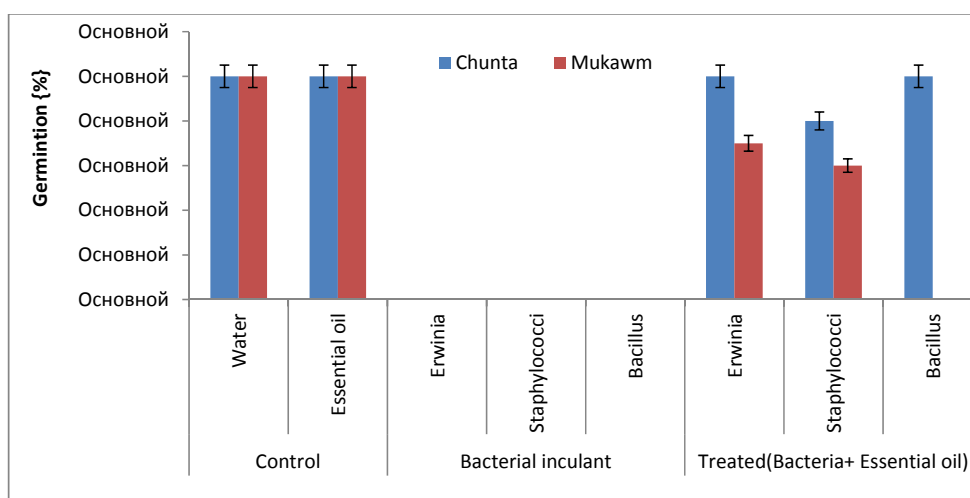


Figure 2 – Percentage of two varieties of wheat grains germination in controls and different variants contaminated with *Erwinia*, *Staphylococci* and *Bacillus*

Although among the bacterial inoculated variant on four varieties of wheat seeds had result that shown the resistance of wheat variety against inoculated bacteria such as *Pseudomonas* inoculant on Ghory and Sulh varieties, these cultivars could germinate and growth by a little different with control, the first day after germination respectively,

i. e. 3,5 and 5 cm but in the inoculated 2 and 2,5 cm grew but the treated variants were satisfied because they had the same length like controls variants but a little different in *Pseudomonas* contaminated, i.e. the first day 5,5 and 5 cm treated *Pseudomonas* inoculated and in the controls 3,5 and 5 cm (Figure 4).

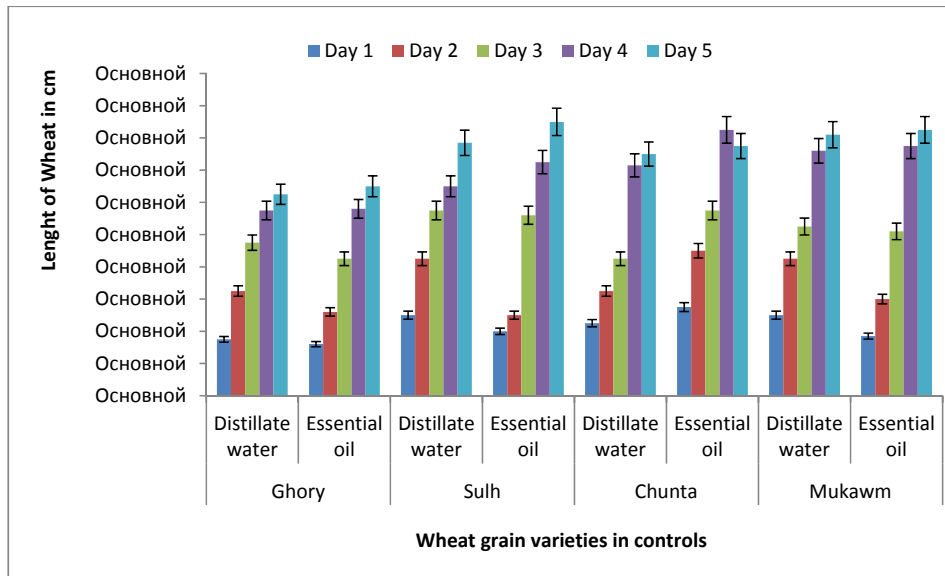


Figure 3 – Growth of four wheat varieties as controls during five days

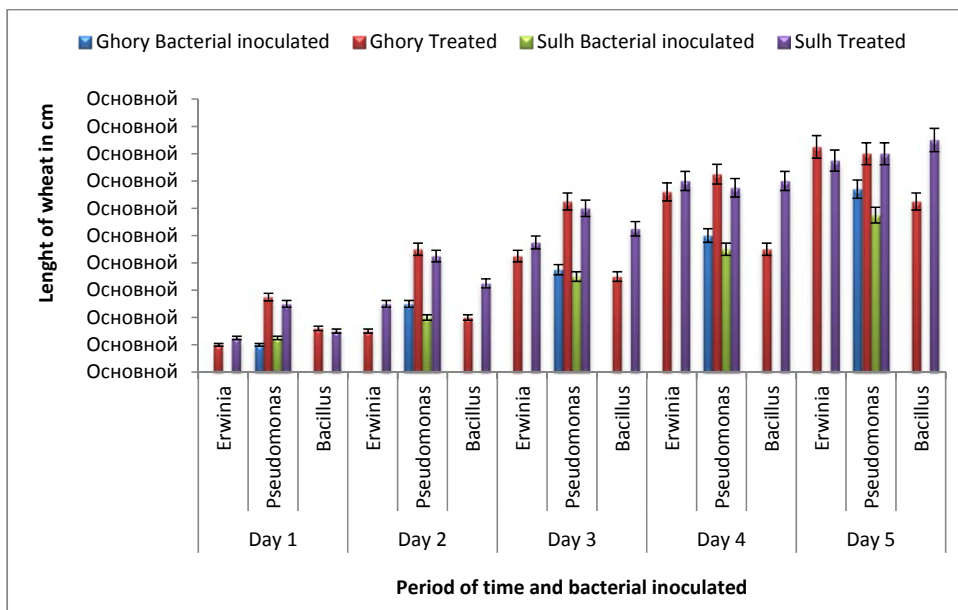


Figure 4 – Growth of Ghory and Sulh varieties in treated and inoculated during five days

But the treated variants had greatly growth Chunta variety that had the highest growth among the inoculated bacteria especially contaminated by *Bacillus* then *Staphylococci* and *Erwinia* treated varieties, so in the bacterial inoculated variant fully eliminated all cultivars these bacteria colonized and produced black spots the covered the wheat seeds. Thus the elimination of seeds by colonizing of bacteria revealed these bacteria enable to prevention of germinating and finally destroy the yield (Figure 5).

In fact the growth of treated variants got speed after third day of germination, so the essential oils variants grow up day by day with hadn't any difference, it replayed the essential oils of hyssop affected on bacterial inoculant during first until third day of germination and then support the growth process of wheat. And the all cultivars were susceptible against all genera such as *Pseudomonas*, *Erwinia*, *Bacillus* and *Staphylococci* but only two varieties like Ghory and Sulh were resistance against *Pseudomonas*.

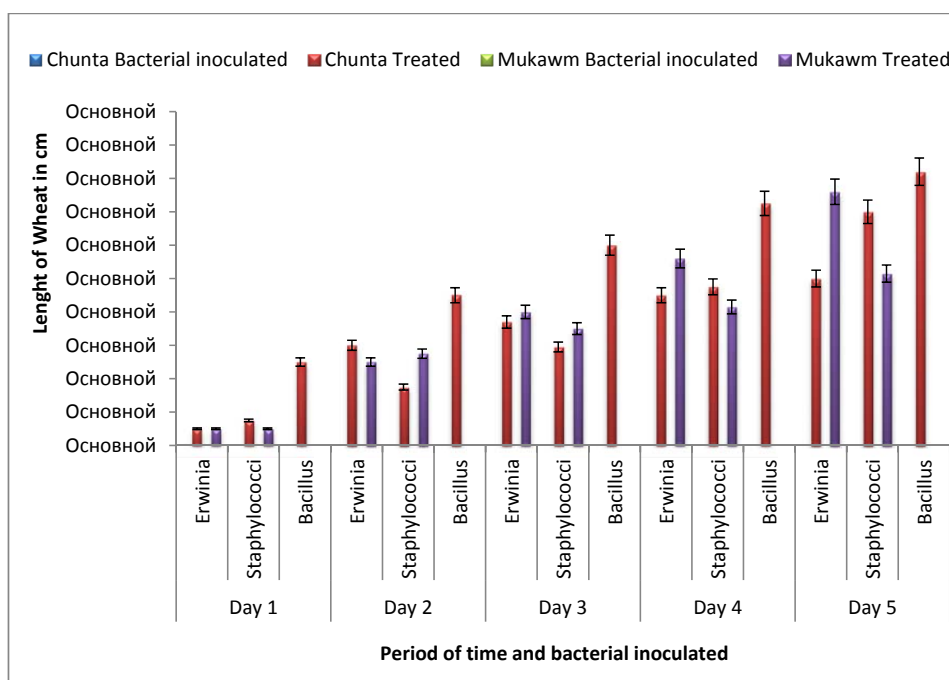


Figure 5 – Growth of Chunta and Mukawm varieties in treated and inoculated during five days

Conclusion

This study received among which bacteria were sensitive against essential oils of hyssop, although they had difference level effecting such as *Bacillus* the highest sensitive versus essential oils of hyssop in all varieties of wheat seeds successfully controlled and the growth of wheat seeds lengths the same in controls variant in case the inoculated variant eliminated the wheat seed for example in Chunta variety at the control was grown during five days like 4,5cm 6,5cm 8,5cm 14,3cm and 15cm but on the treated variant also was grown at the period of five days that consist

respectively as 5cm 9cm 12cm 14,5cm and 16,4cm. The *Erwinia* and *Staphylococci* genera also were sensitive but not like *Bacillus* in Chunta and Mukawm varieties.

Thus revealed inoculated bacteria were very sensitive against essential oils of hyssop and the extraction of essential oils of hyssop is not very complex also by using of this compound we can prevent of environmental pollution and other hazard of using chemical compound that causing resistance like bacteria via conjugation or transformation, only we need to add 10.2 ml/lit of essential oil in distillate water and inoculate the wheat seeds with this solvent before cultivation.

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