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Effects of Estrogen in Birth Control Pills on Germination and Growth in Grass Pea (*Lathyrus sativus* L.)

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Abstract

The aim of this study was to assess the effects of combined Oral Contraceptive pill, on morphological characteristics and parameters of biomass production, which includes fresh and dry matter weight (FMW and DMW, g/plant), root and shoot length of Grass pea seeds (*Lathyrus sativus* L.). The combined oral contraceptive pill (COCP), is a type of birth control that is designed to be taken orally by women. It consists of a combination of an estrogen [generally Ethinylestradiol (C₂₀H₂₂O₂)] and a progestogen (specifically a progestin). They're currently utilized by extra than one hundred million women worldwide. This review checked out studies that as compared pills with 20 µg Ethinylestradiol. Plants were grown hydroponically and three different doses of Ethinylestradiol, 0.0 mgL⁻¹ (control), 0.1mgL⁻¹, and 0.2 mg L⁻¹ had been imposed on 2 days after sowing for 28 days' duration. Experimental plots have been set up in a randomized block design with three replications. Seed germination, The Root Length (RL); Shoot Length (SL); Fresh Matter Of Roots (FMR); Fresh Matter Of The Shoot (FMS); Dry Matter Of The Roots (DMR); Dry Matter Of The Shoot (DMS) have been decided. Morphometric parameters of roots have estimated the usage of an image analysis software program. Root length (RL) and specific root length (SRL) have been calculated. The growth and biomass response of Grass pea have been more suitable due to adding on Ethinylestradiol. Results showed that Ethinylestradiol in concentrations discovered in sewage water can affect the vegetative boom of Grass pea.

Keywords: Ethinylestradiol; natural environment; Grass Pea; COCP; Water pollution.

Abbreviations: COCP: Combined Oral Contraceptive Pill; RL: Root Length; SL: Shoot Length; FMR: Fresh Matter of Roots; FMS: Fresh Matter of Shoot; RMD: Dry Matter of the Roots; DMS: Dry Matter of the Shoot

Introduction

Pollution of essential water sources has become extreme trouble across a great deal of the world [1]. The specific contaminants main to pollution in water consist of a wide spectrum of chemicals, pathogens, and physical changes including elevated temperature and discoloration. Whilst most of the chemical substances and physical changes which might be regulated can be naturally occurring (calcium, sodium, iron, manganese, and so on.) the attention is regularly the key in determining what a natural aspect of water is and what a contaminant is. High concentrations of naturally going on materials will have

negative effects on aquatic flora and fauna [2]. Depending at the degree of eutrophication, subsequent negative environmental outcomes which include anoxia (oxygen depletion) and extreme reductions in water quality can also occur, affecting fish and other animal populations [2]. One example of water pollution due to human beings is dumping waste merchandise as effluent into most important rivers and waterways. Thus far, the greatest quantity of waste that finally ends up in marine ecosystems is sewage [3]. Sometimes naturally happening chemical substances, including hormones, also can increase in these aquatic environments because of waste effluent. One of the most not unusual estrogenic resources in wastewater effluent

comes from the urine of women who take birth control pills. Some birth control tablets incorporate hormones estrogen and progesterone. Those hormone treatments incorporate artificial ethinylestradiol in addition to significant quantities of 17 β -estradiol [4]. Ethinylestradiol (EE) is an estrogen medication which is used extensively in birth control tablets in mixture with progestins. It's also every now and then used as a component of menopausal hormone remedy for the remedy of menopausal signs and symptoms in combination with progestins [5]. In mammals, the steroid intercourse hormones play a key role in controlling the methods of improvement and reproduction and they're additionally engaged inside the control of mineral and protein metabolism [6]. Mammalian sex hormones together with estrogens, androgens, and progesterone belong to steroids, a collection of compounds which have a simple sterane carbon skeleton. The different steroids in residing organisms are decided by the location and styles of useful groups connected to the sterane (Figure 1). Steroid sex

inhibited root growth, however, root elongation became promoted through progesterone on the attention 0.1 μg per plant. Testosterone promoted cotyledon axillary bud formation at concentrations of 0.1 and 0.25 μg per plant [8]. In tomato seedlings, estrone and 17 β -estradiol (as sulfate derivatives, on the attention of 1 μM in nutrient solution), decreased root growth in addition to root number in shoot cuttings [16]. Research carried out on varying species of plants established that β -estradiol reduced the germination percent of lettuce, carrot, and tomato seeds (*Lactuca sativa* L., *Daucus carota* L., and *Lycopersicon esculentum* Mill. respectively). Germination turned into decreased by 57% in *L. Sativa*, 6% in *D. carota*, and 18% in *L. esculentum* when in comparison to the controls [17]. The examiner carried out on chickpea seeds (*Cicer arietinum* L.) ended in a dramatically extraordinary set of results. Each β -estradiol and progesterone improved seed germination. After forty-eight hours seed germination extended from 85% inside the control to 100% within the β -estradiol handled group [18].

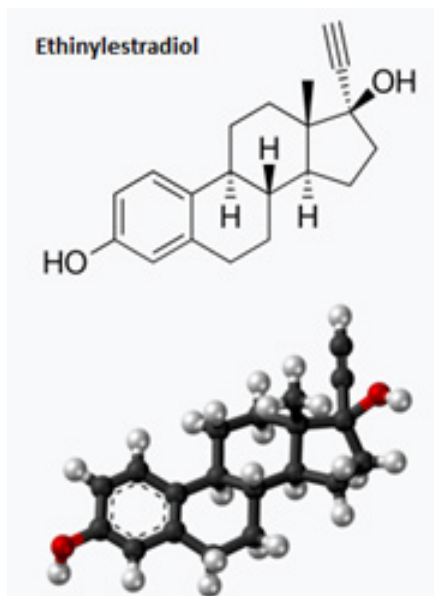


Figure 1: Ethinylestradiol.

hormones implemented exogenously (e.g. progesterone, estrone, β -estradiol, testosterone) stimulate growth and improvement (callus proliferation, cell division, root and shoot elongation, pollen germination flowering) [7-12]. The impact of exogenous mammalian sex hormones on plant increase at the beginning of the twentieth century, it turned into shown that estrone inspired the growth of an isolated pea embryo in-vitro [13,14]. Estrone, at the awareness of 0.1 μg per plant, additionally stimulated the growth of *Pisum sativum* L. seedlings by approximately forty% [15]. In sunflower seedlings, 17 β -estradiol and progesterone (0.25 μg in step with plant) extended shoot increase but

Grass pea (*Lathyrus sativus* L.) is an annual crop and associated with the Fabaceae (Leguminosae) genus. In archeological unearthings in Turkey and Iraq, seeds of *Lathyrus* species had been found as collected or developed things. So also, seeds from 2500 bc were recognized within the most seasoned excavations in India [19] and as of now within the Balkan in 8000 bc. Agreeing to the legend, grass pea was brought to Ethiopia (Abyssinia) by the queen of Sheba after going by Lord Salomon within the tenth century bc [20]. Grass pea presents a fascinating paradox, it's far both a lifesaver and a destroyer. It's far without difficulty cultivated and might face up to extreme environments from drought to flooding because of this hardiness, grass pea is regularly the only alternative to starvation while other crops fail [21]. With the massive environmental motion that ushered within the millennium, scientists started to take any other look at the role that contaminants play in the environment and specifically started to study their consequences on plant life. Cutting-edge research has focused on the consequences of these contaminants on vegetative plant growth [1]. The purpose of this research was to investigate the effects of estrogen in birth control pills on Germination and growth in grass pea (*Lathyrus sativus* L.)

Material and Methods

This study was carried out at the research centre for Plant Sciences, Ferdowsi University of Mashhad, Mashhad, Iran. It was carried out in a laboratory under the hydroponic situations on the ambient temperature. Seeds of grass pea have been from the seed bank of research centre for Plant Sciences, the Ferdowsi University of Mashhad (Figures 2-4).



Figure 2: Grass pea (*Lathyrus sativus* L.).

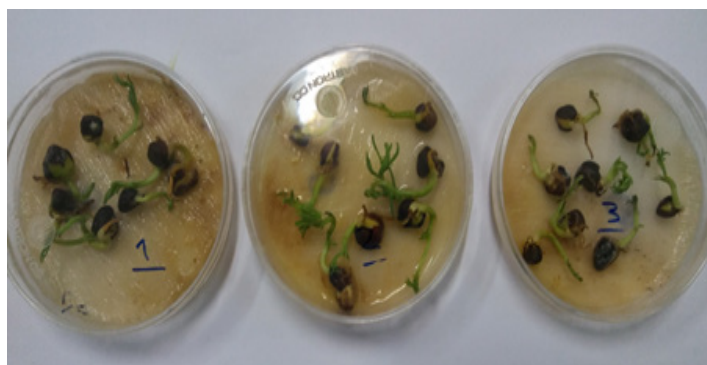
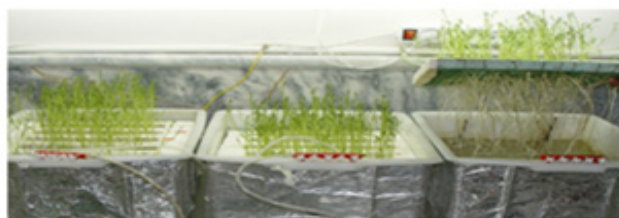


Figure 3: Eight Grass pea (*Lathyrus sativus* L.) kernels were placed into petri dishes lined with filter paper. The dishes were then randomly assigned a treatment group of various Ethinylestradiol concentrations (0.0 mgL^{-1} (control), 0.1 mgL^{-1} , and 0.2 mg L^{-1}).

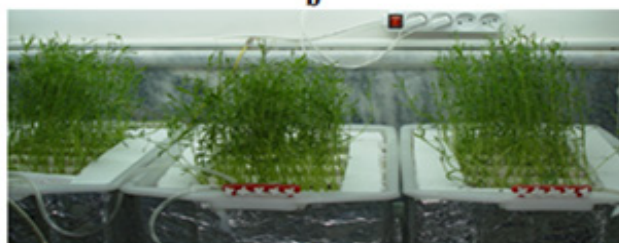
All of the seeds have been first checked for their viability with the aid of suspending them in deionized water. The seeds which settled to the bottom have been decided on for in addition study. Seeds were sterilized in a 5% sodium hypochlorite solution for 10 mins [22], rinsed via with deionized water several times. The birth combined oral contraceptive pill (COCP) became dissolved first in a small quantity of Distilled water after which diluted in water that allows you to obtain the subsequent concentrations: 10 and 15 M. plants had been transferred to plastic containers (20 L) with a nutrient solution Nutrient solutions (Hoagland solution) have been constantly aerated with the aid of an air-pump to maintain it oxygenate and to preserve homogeneous the solution. The Hoagland solution is a hydroponic nutrient solution (Table 1) that changed into developed by using Hoagland and Arnon in 1950 and is one of the most famous solution compositions for growing plants. The Hoagland solution provides every nutrient vital for plant growth and is appropriate for the increase of a huge variety of plant species [23]. Every treatment became replicated three times. The nutrient solution turned into renewed weekly and the containers have been watered if necessary. Plants have been sampled after 28 days of



a



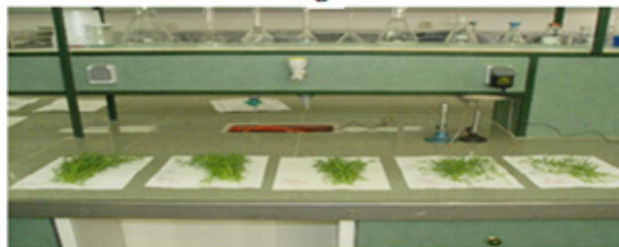
b



c



d



e

Figure 4: Effect of Ethinylestradiol applications on Grass Pea root growth. Seedling day 7 (a) Root and Shoot length of 28-day old control (b) and Ethinylestradiol (c) treated. Number of lateral roots in 28-day old, Ethinylestradiol (d). Representative image of the Shoot in 28-day old, control and Ethinylestradiol treated (e).

treatment. For the germination tests, Semipermeable paper (Whatman No. 2) was put in standard scale Petri dishes (100 mm × 15 mm). Eight seeds were set equally over each paper and secured with another layer of Whatman No.2-

channel paper forming a sandwich. Five mL of the proper birth combined oral contraceptive pill (COCP) arrangement was added to each Petri dish utilizing Air displacement micropipettes. Following the situation of the seeds on the filter paper, the filter papers were soaked with the appropriate test saline solutions. The temperature during germination was held at 25 ± 1 °C. Three reproduces of each testing condition have been accommodated investigation.

Roots and shoots were separated and weighed. Roots were thoroughly rinsed in the faucet and distilled water. Then both shoots and roots have been rinsed in distilled water for two min. determination of Root Morphology At day 28, root length (cm) and root surface vicinity (cm²) of Grass pea were determined using a root scanner, Delta-T test (model D55/151). Plant material changed into dried at 60 oC for seventy-two h. Parameters Measurer/ Shoot length (mm), shoot dry weight (mg), root dry weight (mg), total dry weight (mg), (all dried at 80 oC for forty-eighth). The following relationships have been derived: the ratio of shoot dry weight/root dry weight, ratio of stem dry weight to stem length. Leaf proline substance was evaluated according to the strategy of Bates et al. [24] from completely extended leaf tests collected from to begin with shaped essential branches on particular collect dates. SPSS Ver.16 was used for comparing the means the use of the Duncan test at $P < 0/05$, level of significance [25].

Results and Discussion

The goal of this experiment was to determine if Water major pollutants, combined Oral Contraceptive, had an effect at the growth of grass pea (*Lathyrus sativus* L.) seedlings. Grass Pea plants have been grown in the absence or presence of the Combined Oral Contraceptive (Ethinylestradiol), under various conditions. analysis of the effect of various amounts of Ethinylestradiol at the Grass Pea attention showed a significant difference between experimental groups, control, and case groups $P < 0/05$. Data presented in (Table 1) indicated that seed germination was significantly affected by lower and highest Ethinylestradiol and progesterin concentration in the culture medium (0.1 and 0.2).

At smaller concentrations, it appears that Ethinylestradiol causes a slight escalation in overall germination, 97% in 0.1 mg when compared to the control at 92%. Germination is a key phase in the initiation of plant growth. At the highest concentration of the hormone, 0.2 mg/L, the overall germination was reduced to 73%. This indicates that at some level Ethinylestradiol is toxic to the kernels at high concentrations. If germination is being inhibited by high concentrations of the hormone, this could indicate that

seed sown in fields that are exposed to contaminated water or soil, may have reduced germination rates and therefore reduced overall yield. These results seem to fall in line with the plant species *Lactuca sativa* L. and *Lycopersicon esculentum* Mill. (Tomato) which also exhibited a reduction in the overall rate of germination when exposed to β -estradiol [17]. The growth of shoots and roots was also negatively affected by Ethinylestradiol in studied Grass pea (0.2 mg L⁻¹). The average increase for the Grass Pea plants that received combined oral contraceptive pill (COCP) was 51 cm as compared to 28 cm for Grass Pea plants that did not receive estrogen. After 42 days of cultivation in medium containing 0.2 mg L⁻¹ Ethinylestradiol, the reduction of shoot length ranged from 3.8% to 34.4% compared to the non-treated control. In mammals, β -estradiol plays a key role in controlling the processes revolving round improvement and reproduction in addition to being worried about the control of both mineral and protein metabolism [1]. Numerous researches was carried out on different plant species to be able to determine this.

In sunflower seedlings (*Helianthus annuus* L.), β -estradiol concentrations of 1 μ g according to plant increased overall shoot growth however had been shown to inhibit general root growth [11]. In chickpeas (*Cicer arietinum* L.), the hormone considerably enhanced the root and shoot growth of the seedlings at concentrations of 10⁻⁴, 10⁻⁹, 10⁻¹², and 10⁻¹⁵ M [18]. Among the growth traits, plant height, the number of primary branches, and leaves per plant reduced markedly in Grass Pea seedlings subjected to 0.2mgL⁻¹ but increased markedly in Grass Pea seedlings subjected to 0.1mgL⁻¹. According to the one factor ANOVA analysis, there's a distinction in effect that Ethinylestradiol concentration and the controls had on the Grass Pea plants. Treatment with Ethinylestradiol in, 0.1 mg⁻¹ (increases both shoot and root dry weight). Plants exposed to higher concentrations of Ethinylestradiol had significantly lower root growth, Increasing the Ethinylestradiol concentrations in a medium led to a decrease of root growth between 68.8% in Grass pea, The highest level of Ethinylestradiol (0.2 mg L⁻¹) decreased the root length between 88.3% and 97.8% compared to the control treatment (0 0.2 mg L⁻¹), indicating a toxic effect of Ethinylestradiol. Increasing Ethinylestradiol concentration in the media the weight of the plants significantly decreased from 16.5% at 0.1 mg L⁻¹ Ethinylestradiol to 89.5% in 0.2 mgL⁻¹ Ethinylestradiol. It can be concluded that Ethinylestradiol in concentrations discovered in sewage water can have an effect on the vegetative growth of Grass Pea plants Ethinylestradiol application had significant effects on shoot diameter and number of leaves. Mean shoot and root lengths numerous

Table 1: Concentration ranges of essential mineral elements Hoagland & Arnon.

Nutrient	Mg L ⁻¹
N	210
P	31
K	234
Ca	160
Mg	34
S	64
Fe	2.5
Cu	0.02
Zn	0.05
Mn	0.5
B	0.5
Mo	0.01

Table 2. Effect of Ethinylestradiol application on biomass accumulation and growth parameter of Grass pea

Data are means \pm standard error of a least three replicates, Means followed by common letters are not significantly different ($P < 0.05$, ANOVA); FW= Fresh weight; DW= Dry weight

Treatment	Control	0.1mgL ⁻¹	0.2 mg L ⁻¹
Seed germination	86.3a	93.3a	42.9b
Primary branches/ plant	13.52a \pm 0.16	16.14b \pm 0.11	5.67c \pm 0.15
Root length (mm)	18a \pm 24.7	27.3a \pm 12.1	8.7c \pm 7.4
Shoot length (mm)	45.8a \pm 11.4	55.2b \pm 3.3	17.7c \pm 8.6
Root DW (gr/plant)	0.21a \pm 0.33	0.25a \pm 0.17	0.06b \pm 0.04
Shoot DW (gr/ plant)	1.16a \pm 0.28	1.85a \pm 0.12	0.75b \pm 0.14
Leaf Proline Content $\mu\text{mol} / (\text{grFW})$	2.64a \pm 0.53	2.75b \pm 0.61	3.55c \pm 0.85

significantly according to Ethinylestradiol application (Table 2). Ethinylestradiol inoculation additionally resulted in a higher mean number of leaves per plant (Table 2). Among the parameters responding to Ethinylestradiol treatment, rapid accumulation of free proline content is one of the significant events in plants. In grass pea, leaf proline content increased with increasing exposure to Ethinylestradiol (Table 2).

Conclusion

worldwide, steroid estrogens including estrone, estradiol, and estriol, pose severe threats to soil, plants, water resources, people and have gained outstanding interest in current years, because of their rapidly increasing concentrations in soil and water all over the world, and the problem has been expressed concerning the access of estrogens into the human food chain which, in turn, relates to how plants absorb metabolism estrogens [26]. Estrogens and estrogen-like compounds (xenoestrogens) from livestock manure, animal waste, and human waste (especially pharmaceutical waste), are being disposed of and excreted at high charges into the agricultural soil and groundwater all around the international [27]. Hence, in the gift study, we aimed to search the effect of the combined oral contraceptive pill (COCP) on morphologic (roots and stem length) parameters in Grass Pea. To the excellent of our knowledge, there has not been any report at the effect of mammalian sex hormones on morphologic (roots and stem length) in Grass Pea seeds, one of the most critical legume crops of the world. Due to the fact, there isn't always sufficient research and knowledge on the effects of combined Oral Contraceptive pill in plant life, its miles necessary to achieve advanced researchers on biochemical parameters.

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