

AN INVESTIGATION ON THE EFFECT OF HORMONE POLLUTION ON SURVIVING PLANT LIFE

Research Question: To What Extent Do Varying Concentrations of a Combined Estrogen and Progesterin Pill Solution (0.25, 50, 75, 100 mg) Versus a Progesterin-Only Mini Pill Solution (0.25, 50, 75, 100 mg) Impact the Biomass of the *Phaseolus vulgaris* Plant During a Five-Day Growth Period?

Hormone Pollution and The Environment

According to a report in 2018 by The United Nations Educational, Scientific and Cultural Organization, UNESCO, 80% of wastewater finds its way back into the ecosystem without proper treatment or being reused for other purposes in a global trend. (WATER QUALITY AND WASTEWATER, 2018)

Natural hormones such as Testosterone and Estrogens from both humans and animals are excreted into the environment. These steroidal hormones are naturally found in water bodies and sewage used for irrigation, and even in smaller concentrations after vigorous treatment. (Olowu and Okunribido, 2016)

A study conducted in Chamosca city, Argentina where estrogen, androgens, and progesterone were noticed in wastewater treatment plant used to obtain results. The natural hormones which were found included: 17 β -estradiol (E2), estrone (E1), testosterone (T), 17 α -dihydrotestosterone (DHT), progesterone (P), 17 β -hydroxyprogesterone (17OH) and the synthetic estrogen, 4-ethyltobacetyl (EED). This study also signifies the presence of steroidal hormones and estrogenic activity in water and suggests the presence of a source, or multiple, for these findings. (García et al., 2020)

17 β -estradiol was found in the wastewater outlet and receiving water, which is also one of the steroidal hormones used in a study by Laurence & Shore in which he investigated the vegetative growth of alfalfa, *Medicago sativa*, after being exposed to estrogen and, as earlier mentioned, 17 β -estradiol. In this study, Shore states that the growth curve showed that the growth of the shoot and roots of the *Medicago sativa* was increased in exceedingly low concentrations of estrogen; however, when dosage was increased from the range of 0.003 to 0.3 μ g/l it was the way up to concentrations of 50 to 500 μ g/l the growth curve showed a decrease in overall growth of the alfalfa plant. (Olowu et al., 1992)

What Are Steroid Hormones?

Steroid hormones can be split into two groups, corticosteroids and sex steroids.

Corticosteroids are released by the adrenal cortex and include hormones such as cortisol, involved in stress response, and aldosterone, which is essential to sodium conservation in multiple parts of the body.

The other group being is sex steroids, produced by the ovaries or testes, or the placenta. Sex hormones include different types of estrogens, androgens, and progesterone.

Hormonal pharmaceuticals typically include two forms, a combination pill, which contains a type of estrogen and a type of progesterin, and a mini pill, which contains a type of progesterin only. Just as hormones in human urine are elevated during pregnancy, they are also elevated when a woman is taking birth control. 151 million women worldwide use some form of oral contraceptive (United Nations, 2019).

All these excess hormones are released into wastewater which may then be released back into the environment.

What Pills Were Used in This Investigation

Yaz contraceptive pills contain a combination of two hormones, ethinyl estradiol (estrogen) and drospirenone (progesterin). This birth control is mainly used to prevent unwanted pregnancy, however, it may also be used to aid with menstrual pain, reduce acne, and prevent ovarian cysts and aid in premenstrual dysphoric disorder (PMDD). Each packet contains 24 pills that contain sex hormones and 4 remainder pills without hormones. Yaz contains 1mg of drospirenone, which has antiandrogenic activity.

Ultracetin is a type of micronized progesterone which can be used in hormone replacement therapy (HRT) to relieve menopause symptoms. A usual dose of Ultracetin (two pills a day), each pill contains 100mg of progesterone

Aim and Hypotheses

The aim of this study is to determine whether the biomass growth of plants is inhibited by hormones, and how different concentrations of these hormones affect growth.

A rise in the concentration of steroidal hormones and other chemical waste in water bodies, caused by contamination through water waste accumulated by human use (Olowu and Okunribido, 2016), can potentially cause stress to surrounding plant life and inhibit growth and affect hormone concentration of plants.

This water may come into contact with plants we use for our sustenance, such as beans, and largely affect our diet and the future along with causing a noticeable change in the diet and livelihood of wild animals. (Olowu et al., 1992)

Therefore, it would be fair to assume that the growth of *Phaseolus vulgaris* would be negatively affected at higher concentrations of steroidal hormones from oral contraceptives.

Null Hypothesis (H0): There is no significant correlation between the mammalian steroid hormones and the biomass growth of green bean (*Phaseolus vulgaris*) plants under any condition when compared to controls.

Alternate Hypothesis (H1a): There is a significant correlation between the mammalian steroid hormones and the biomass growth of green bean (*Phaseolus vulgaris*) plants when compared to controls. There is a more severe lack of growth in samples when exposed to the higher concentrations of hormones during the treatment period.

| Independent Variable | Units | Effect | Dependent Variable | Units | Method of Measurement |
|--|---------------------------|--|-----------------------------|-------|--|
| Concentrations of the Ultracetin pill (0.25, 50, 75, 100 mg) | mg (0.25, 50, 75, 100 mg) | Due to the effect of high concentration of progesterone, it can be concluded that higher concentrations will have a higher impact on the growth of the plant. | The biomass of each plant | g | Changes in circumference, weight, and height of the plant were taken at the beginning, middle, and end of the experiment. To avoid measuring every part of each plant everyday, the growth was measured during the growth period to give an overall biomass. |
| Concentrations of the Yaz pill (0.25, 50, 75, 100 mg) | mg (0.25, 50, 75, 100 mg) | Due to the effect of high concentration of estrogen and progesterone, it can be concluded that higher concentrations will have a higher impact on the growth of the plant. | Overall biomass of 3 plants | g | Each plant was weighed on a scale to give an overall biomass. |

Controlled Variables

| Variable | Unit | Possible Effect | Method of Control |
|-------------------------------|------|---|---|
| Concentration of estrogen | N/A | May cause stress to the plant and inhibit growth. | Use the same amount of estrogen in all treatments. |
| Concentration of progesterone | N/A | May cause stress to the plant and inhibit growth. | Use the same amount of progesterone in all treatments. |
| Amount of distilled water | ml | May kill the plant if not enough water is added. | Use the same amount of distilled water in all treatments. |
| Amount of distilled water | ml | May kill the plant if not enough water is added. | Use the same amount of distilled water in all treatments. |
| Temperature | °C | Temperature greatly affects the growth of plants. High temperatures can cause stress to the plant and inhibit growth. | Use the same amount of distilled water in all treatments. Use the same amount of distilled water in all treatments. Use the same amount of distilled water in all treatments. |

Preliminary Work

Disinfecting Pills

To attempt to dissolve this capsule solution had to be found that would not harm the plants; originally an organic option was attempted and apple cider vinegar was used to try and dissolve the pill. Apple cider vinegar to distilled water concentrations were made: 1ml (100ml), 5ml (95ml), 10ml (90ml), 15ml (85ml), 20ml (80ml), 25ml (75ml). The amount of apple cider vinegar in each solution was kept relatively low so as not to kill the plants.

After a five-day growth period, it was found that none of the seeds in the petri dishes containing apple cider vinegar had germinated, meanwhile, the petri dishes with pure distilled water showed significant growth. Due to the lack of germination apple cider vinegar was removed. In addition, it was observed that the apple cider vinegar would evaporate exceedingly fast, causing a need for extra watering, making disposal and cleaning of the petri dishes for the experiment.

An earlier experiment on the effects of mammalian sex hormones on chickpeas plants used small amounts of methanol to dissolve progesterone, testosterone, and androstenedione which were then diluted in water to give multiple concentrations. (Erdal and Daulakovic, 2016)

After the five-day growth period it was noted that while the control, treated with exclusively distilled water, had shown largest growth in biomass, the solutions containing 1ml and 10ml of methanol proved to also show significant and similar growth patterns. However, a concentration of methanol of 15ml or above showed signs of growth being stunted. Due to contraceptive pills not requiring large amounts of methanol to dissolve, the 5ml-95ml solution of methanol and distilled water was chosen for the final experiment.

Testing Germination

Although the apple cider vinegar did not allow for the germination of seeds, it was tested if the seeds could germinate with distilled water for a three-day period and then be exposed to the apple cider vinegar solutions. After the seeds had germinated, the apple cider vinegar was applied. However, the solutions completely stunted the growth of the seeds.

The same experiment was conducted on the methanol solutions, however the growth in biomass seemed to be nearly the same for the 5ml-95ml solution as for the control whether the seeds were pre-germinated or not, and so, it was decided to not pre-germinate seeds.

Choosing Seeds

All the beginning of preliminary experimentation three plants were tested: Black chick-pea (Cicer arietinum), black beans (*Phaseolus vulgaris*), and green bean (*Phaseolus vulgaris*).

After extensive testing it can be noted that the majority of petri dishes containing Black chick-pea (Cicer arietinum) show signs of mold by the end of the test period even after vigorous sanitisation of equipment. After observing all plants and no other trials made by the end of the test period even after vigorous sanitisation of equipment. After observing all plants and no other trials made by the end of the test period even after vigorous sanitisation of equipment. After observing all plants and no other trials made by the end of the test period even after vigorous sanitisation of equipment.

Black beans and green beans are essentially identical except for the colour of the bean, and show exceedingly similar growth patterns. Due to an abundance of green bean seeds and the apparent lack of difference in growth, green beans were decided upon for the final experiment.

Materials and Method

Materials and Method

1. Making the solutions:

- The amount of pills needed for the different mg amounts of active ingredients/hormone per solution was calculated.
- Some of the pills were needed to be cut into sections, as a whole pill was not always possible. Each 100mg pill, containing 100mg of progesterone, was split. In this experiment, the pills were found to split the liquid inner core and been cut with a sharp knife into 23 mg and 77 mg pieces, ensuring the active ingredients were equally distributed. The Yaz pills were also cut, as the 23 mg portion. This specific solution was determined to require a whole pill and approximately one-third of a pill.
- After the amount of pills was calculated, the pills for each individual solution were ground up using a mortar and pestle.
- 5 ml of methanol, measured using a 2 ml measuring cylinder, was added into the mortar to dissolve the pills better. Some distilled water was then added to increase the fluidity of the mixture and was poured into a 100 ml beaker. The mortar was filled with distilled water to capture any residue from the pills, and the water was poured into the beaker (this was repeated a few times if needed).
- The 100 ml beaker was filled the rest of the way with distilled water until the 100 ml mark was reached.
- This solution was then transferred to a 500 ml beaker which was filled with precision using distilled water in between solutions.
- This process was repeated for every solution. All equipment was rinsed with precision using distilled water in between solutions.
- It should be noted that approximately 84 Yaz combination contraceptive pills were needed for the solutions, as 4 sheets of pills were raised the precision of the measured solutions (methanol, distilled water), so one sheet was sufficient. To go over the measurement limit on the equipment.
- A control was also made, containing 2 ml of methanol and 98 ml of distilled water.

2. Preparing the seeds:

- Nine petri dishes were used to hold the different seed groupings: 4 for the Ultracetin solutions, 4 for the Yaz solutions, and one control.
- Unwashed cotton balls were laid at the bottom of each petri dish to act as a bed for the seeds, similar to soil.
- Five seeds were placed into each petri dish and measured individually on a 0.01g scale before introducing the seeds to hormones so that the growth in biomass of each plant could be measured over time.

3. Procedure during growth period:

- Each petri dish was sprayed with its respective solution on a daily basis.
- The weight of each individual seed was kept track of on a daily basis. The 3-gram petri dish scale was cleaned before, in between, and after weighing seeds. The weights were required to be tracked on an online spreadsheet could be used.
- The seeds were labelled to ensure tracking of each seed individually.

Results: Qualitative Observations

| Initial (day 1) | During (day 3) | After (day 5) |
|--|---|---|
| <ul style="list-style-type: none"> There is five seeds per petri dish All seeds are a muted green colour | <ul style="list-style-type: none"> All seeds have sprouted by this point The sprouts are a light green colour Plants in the lowest hormone concentrations have sprouted leaves Plants in the control have sprouted leaves | <ul style="list-style-type: none"> The plants exposed to higher hormone concentrations show a slight yellowish colour Plants exposed only to solutions of 25mg progesterone have sprouted leaves and leaf growth The high hormone concentrations show signs of underdevelopment when compared to the control |

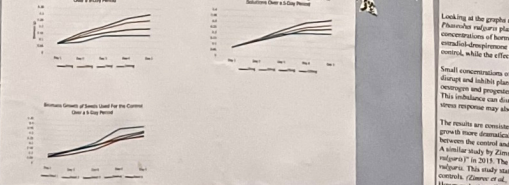
Litrogesterin Pill Solutions (Biomass in Grams)

| Ultracetin Solution | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---------------------|--------|-------|-------|-------|-------|
| Seed 1 | 0.25 | 0.15 | 0.20 | 0.25 | 0.22 |
| Seed 2 | 0.07 | 0.15 | 0.21 | 0.20 | 0.23 |
| Seed 3 | 0.02 | 0.12 | 0.19 | 0.26 | 0.31 |
| Seed 4 | 0.074 | 0.103 | 0.20 | 0.247 | 0.289 |
| Seed 5 | 0.083 | 0.131 | 0.262 | 0.312 | 0.341 |
| Averages | 0.0748 | 0.118 | 0.236 | 0.312 | 0.34 |

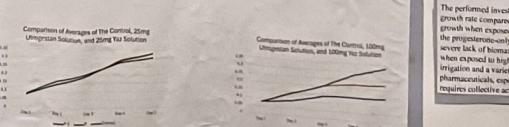
| Yaz Solution | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|--------------|--------|-------|-------|--------|-------|
| Seed 1 | 0.02 | 0.14 | 0.24 | 0.292 | 0.33 |
| Seed 2 | 0.03 | 0.15 | 0.24 | 0.216 | 0.219 |
| Seed 3 | 0.07 | 0.139 | 0.202 | 0.202 | 0.22 |
| Seed 4 | 0.078 | 0.145 | 0.189 | 0.229 | 0.23 |
| Seed 5 | 0.07 | 0.124 | 0.179 | 0.185 | 0.211 |
| Averages | 0.0774 | 0.144 | 0.208 | 0.2084 | 0.225 |

| Average of All Ultracetin Pill Solutions | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|--|--------|-------|-------|-------|-------|
| 25mg | 0.0748 | 0.118 | 0.236 | 0.312 | 0.34 |
| 50mg | 0.0776 | 0.128 | 0.232 | 0.273 | 0.3 |
| 75mg | 0.077 | 0.134 | 0.204 | 0.206 | 0.226 |
| 100mg | 0.074 | 0.142 | 0.203 | 0.204 | 0.225 |

Graphs of Averages



Comparison Graphs



Analysis and Discussion on Results

Looking at the graphs and raw data, comparing the concentrations of combination pill and mini pill solutions to the control and growth in biomass of *Phaseolus vulgaris* plants, several relationships become apparent. The alternate hypothesis is proven as there is a correlation between the concentrations of hormones and the lower growth in biomass of the sample plants, regarding both conditions progesterone and estrogen response may also be heightened, which may affect the growth of plants.

Small concentrations of hormones can promote plant growth, but at high concentrations such as these, it becomes apparent that mammalian hormones disrupt and inhibit plant growth. Plants and animals have their own respective hormonal systems. While mammalian steroid hormones, such as progesterone and testosterone, help regulate the physiology of animals, they do not have a direct role in plants and can disrupt their hormonal balance, which may affect the growth of plants.

The results are consistent with other studies such as Shore et al., 1992. Shore's study found that higher concentrations of hormones decreased plant growth more dramatically when compared to the control, which is consistent with the biomass growth decrease shown when observing the plant growth. A similar study by Zaremski et al. explored the effects of progesterone and estrogen on germination and growth of Common Bean (*Phaseolus vulgaris*) in 2017. The study found that hormone concentration above 50 significantly inhibits the onset and development of the *Phaseolus vulgaris*. This study states that plants exposed to high hormone concentrations typically show a 40% decrease in biomass when compared to the control. (García et al., 2015)

However, both studies mention that at very low hormone concentrations biomass may appear similar to the control or even increased in some cases.

Conclusion

The performed investigation shows that seed samples of *Phaseolus vulgaris* exposed to mammalian sex hormones have a decreased growth rate compared to samples in a solution devoid of hormones. With both drugs tested, there was a tangible and severe lack of growth when exposed to the highest concentrations (100mg), which affirms the alternate hypothesis stated in the investigation. However, the progesterone-only mini pill, Ultracetin, solutions show a similarity to the control at the start of the 5-day treatment period if a beta when exposed to higher hormone concentrations throughout when compared to the combination pill, Yaz. The experimental decrease in growth potential and a variety of ecosystems and how this may affect surrounding plant life and the importance of this investigation in relation to hormone pollution in pharmaceuticals, especially in the female healthcare industry, and combined a growing environmental concern in today's world which requires collective action.