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Article

Combined progestin-estrogenic contraceptive pills may promote growth in crop-plants

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SUMMARY

Recently, there has been an increase in abundance of chemicals in the environment, especially from therapeutic usages. The effects of these deposited chemicals on the environment and human functions such as the endocrine system are of growing concern. A comparative study was conducted of the combined-effects of ethinyl estradiol and the progestin norgestrel commonly present in contraceptive tablets on the growth of flowering plants. The dicotyledon *Vigna radiata* (mung bean) and monocotyledon *Triticum aestivum* (winter wheat) were treated with progestin-estrogenic contraceptive tablets at 0.150%, 0.300%, 0.450%, 0.600% w/v using distilled water as a control over a period of 6 days. Morphological growth (percentage germination, embryonic and adventitious tissue proliferation, root length, and shoot length) was measured and chlorophyll concentrations were calculated using Arnon's equation for each group. Morphological growth was highest in *V. radiata* treated with the 0.150% solution and in *T. aestivum* treated with the 0.450% solution. Maximal inhibition of morphological growth was observed at 0.600% in *V. radiata*. Progestin-estrogenic contraceptive tablets enhanced morphological growth of *T. aestivum* at all experimental groups compared to the control. Calculated chlorophyll concentrations were higher than the control group at all experimental conditions for both the crop-plants. Maximum chlorophyll concentrations were also found in *V. radiata* and *T. aestivum* treated with 0.150% and 0.450% w/v solutions. While the impact of these chemicals on human health remains unclear, removing these chemicals from the environment is currently not cost-efficient and may either augment or diminish crop yields.

INTRODUCTION

The growth of the pharmaceutical industry and rising population has resulted in increasing amounts of chemicals and their metabolites being deposited in the environment (1-4), contaminating even our drinking water (5). The effects of these pharmaceutical compounds on the environment were seen in 2004 after the mass death of vultures in the Indian subcontinent caused by the anti-inflammatory drug, diclofenac (6). One such class of compounds that has garnered special attention in recent times is gonadal-steroid hormone, i.e. estrogen, progestogen, and androgen.

These hormones are also part of a broader class of compounds called endocrine-disrupting chemicals (EDCs). EDCs are chemicals produced exogenously, which interfere with naturally produced hormones responsible for reproduction, homeostasis, growth, development, and behavior. These EDCs mimic natural hormones and bind to their receptors, interfering in various biological activities such as synthesis, elimination, secretion or transportation of biomolecules (7). Most hormonal EDCs in water bodies exist in the scales of ng/L or µg/L (8, 9). Numerous studies have detected hormones in the sewage, groundwater, and surface water (10-13). Animal livestock feed designed to promote growth are major sources of these hormones (14-16). Another common source of these hormones is the discharge of chemical metabolites from therapeutic usages like contraception and menopausal-hormone therapy after usage by humans. Estrogen and progestin are well-known EDCs (17, 18). Both can be found in contraceptive tablets (19, 20), and feed for animal livestock (21, 22). Every year 30,700kg of natural and synthetic estrogen is discharged from use of contraceptive tablets alone while a further 83,000kg is discharged from livestock globally (23). No such quantifications for progestin have been done to date. When present in water bodies, both ethinyl estradiol and norgestrel reportedly disrupt the hormonal balance of fishes and specifically impede their reproductive system (24, 25). Water from rivers and streams are often directly used for irrigation. In both underdeveloped and well-developed countries, the instruments to detect and remove many of these chemicals are not sustainable (26, 27). Hence, studying the effects of these hormones on crop-plants is crucial.

Ethinyl estradiol is a semisynthetic form of estradiol, which is an estrogen. It binds to the same estrogen-receptor complex and activates similar transcription of genes as natural estrogen (28). It has greater resistance to metabolism than estradiol (29) and therefore, is more susceptible to be egested directly into sewage and not as its metabolites. It is structurally similar to its plant-based counterpart phytoestrogen and binds to the same receptors (30). It is anti-androgenic (31-33). In lower vertebrates like fishes, ethinyl estradiol is heavily involved in feminization (34, 35), and can even affect their trans-generational population sustainability by compromising embryonic survival rate (36). It is usually metabolized by CYP3A4 and CYP2C9 along with a few other isoforms of cytochrome P450 of the electron-transport chain

(37). It's predicted no-effect concentration (PNEC) is derived to be 0.1 ng/L (38). Even though sewage-water is usually passed through sewage treatment plants (STP), removal of ethinyl estradiol is not efficient and often the STP effluent has higher concentrations of ethinyl estradiol than the PNEC (39, 40). It is one of the most common estrogens used in combined oral contraceptives (41). It is almost exclusively used with norgestrel in combined-contraceptive tablets. While the adverse effects of both natural and synthetic estrogens have been studied extensively, studies on the effects of progestin are far less common. Norgestrel specifically has been one of the lesser studied progestins.

Norgestrel is a synthetic (progestin) form of progestogen, containing equal quantities of levonorgestrel which is the active enantiomer, and the inactive isomer dextronorgestrel, making it identical in its biological activity to that of levonorgestrel, but only half as potent (29). However, neither norgestrel nor levonorgestrel has been studied extensively. Most synthetic progestins are structurally similar to progesterone and testosterone (42). Plant-based counterpart of progestins, phytoprogestin is very rare. Progestin, including norgestrel are mediated by nuclear progestin receptors. Levonorgestrel can bind to their androgen receptors in fishes (43). Metabolites of levonorgestrel can exhibit estrogenic activity (44, 45). Progestins are also involved in feminization of fishes (46-48), but a lack of suitable technology to detect the extremely small concentrations of progestin accurately has posed a challenge to detect and derive their PNEC, although concentrations of up to 50 ng/L could be detected in STP effluents (49). A previous study by Fent *et al.* in 2015 compiles the lowest observed effect concentration ranging from 0.8 ng/L to 750 ng/L, on various fishes such as the fathead minnow, zebrafish, roach, etc. These are likely to be true for norgestrel as well since norgestrel has also been suggested to be an active EDC (50). Fates of progestins are not known, but the few studies carried out suggest that they get deposited into sediments and nearby agricultural lands (51). With the possible introduction of progestin-based male birth-control tablets in the future (52), a considerable increase of concentrations in the sewage is likely to be foreseen at this point.

Ethinyl estradiol and progestin work together to inhibit folliculogenesis and ovulation by hindering the mid-cycle surge of luteinizing hormone and the follicle-stimulating hormone (53). These also make the endometrium unsuitable for implantation and thickens the mucus at the cervix (54). The result is contraception.

Ethinyl estradiol and progestins are also responsible for disruptions in plant metabolism (55) although their effects are quite varied. Traces of progesterone were found in mung beans (*Vigna radiata*) (56). In winter wheat (*Triticum aestivum*), estrogen promoted root and leaf growth (57). In wheat, progesterone can promote generative development and induce flowering (58). However, β-estradiol did not cause any significant difference in its seed germination (59). But it decreased the germination rate of *Lactuca sativa*, *Daucus carota*, and *Lycopersicon esculentum* in a separate study (60). Germinated seedlings were measured for their root length and *Daucus carota* exhibited an 11% increase while the other two species had decreased root lengths compared to the control groups.

In another study, 17β-estradiol enhanced shoot growth in *Helianthus annuus* (61). The use of sewage water rather than fresh water for irrigation resulted in better growth in many other plants (62, 63). *Medicago sativa* irrigated with sewage water specifically containing 0.3 μg/L estrogen showed increased vegetative growth (64). Ethinyl estradiol can affect growth and photosynthetic rates in green algae, cyanobacteria (65) and *Arabidopsis thaliana* (66). Progesterone stimulated germination and pollen tube growth in tobacco pollen (67). In chickpea, both progesterone and estradiol enhanced germination velocity, morphological growth and biochemical processes like alpha-amylase, peroxidase and catalase activities among others (68).

V. radiata is a green, dicotyledonous (flowering plant with a pair of leaves, or cotyledons, in the embryo of the seed), leguminous crop-plant, and considered as staple food throughout Southern and Eastern Asia, one of the most densely populated regions. In most other parts of the world it is still a very common food source. Protein is the major form of nutrition found in mung beans (20.97% to 31.32%) (69) with 43.5% of which are essential amino acids (70). These crops are usually planted and harvested before and after cereal crops. Since mung beans are legumes, they can increase the biomass and nitrogen content of soil, acting as green manure (71-73) Mung beans are also well known for their detoxifying bioactivities (74).

T. aestivum is green or brown, monocotyledonous (flowering plant with one leaf, or cotyledon, in the embryo of the seed), grass-like crop-plant, and considered a staple around the world; widely used for cereal production around the world among other major uses. It is vernalized (induction of flowering due to a prolonged period of growth at low temperatures) in the winter. Nutritional benefits include high quantities of carbohydrate, proteins and dietary fiber. Approximately 749 million tons of wheat were produced in 2016 worldwide, second only to maize in terms of the highest production of cereal (75, 76). Although the 13% protein content of *T. aestivum* is relatively low compared to its carbohydrate content (52.4% to 90%) (77), the majority of the protein is in the form of gluten. Lately gluten has been in high demand for its adhesivity and viscoelasticity, which can facilitate the production of processed food (78). In agronomic plants such as wheat, adventitious roots play a significant role in its growth and development (79).

With farmlands and livestock being situated just outside cities, it is possible that the water used to irrigate these farms contain estrogen and progestins. Studying their effects on common crops like wheat and mung beans could be crucial to better farming choices while use of these hormones as potential growth-regulators in these crops to sustain the growing population is another area of interest. A comparative study could also help understand how the effects vary between different species and seed types (mono/dicotyledonous) which have not been studied extensively yet. While effects of individual gonadal-steroid hormones have been studied extensively, studies on the combined effects of these compounds are uncommon but could be of greater significance (80). Hence progestin-estrogenic tablets were chosen as potential growth regulators in this study. The limited scope of the study resulted in selecting tablets containing other accessory substances like their coatings instead of the synthetic hormones in their pure forms. A pilot experiment with two different concentrations of sample solution and distilled water was done on *V. radiata*, which showed varied root and shoot growth over three days. A possible research question including the effect of different concentrations on the growth of plants seemed imminent.

To better understand the combined effects of ethinyl estradiol and norgestrel on morphological growth, root length, shoot length, percentage germination and embryonic leaf and adventitious root proliferation were observed along with changes in chlorophyll concentration, in two different species, one being a monocotyledon (*T. aestivum*) and the other a dicotyledon (*V. radiata*) over six days. In *T. aestivum*, the roots proliferate early on in the germination process before the shoot while in *V. radiata*, it is the opposite. During photosynthesis, chlorophyll b is responsible for the absorption of light and chlorophyll a donates electrons in the electron transport chain. Total chlorophyll concentration can hence be an indirect measurement of the rate of photosynthesis (81), and therefore rate of growth.

Consequently, the scope of this study was to determine the extent to which contraceptive tablets containing the synthetic hormones, ethinyl estradiol and norgestrel (at 0.000%, 0.150%, 0.300%, 0.450% and 0.600% w/v), affect the growth of *V. radiata* and *T. aestivum*. The results suggested that 0.150% w/v sample stimulated the greatest growth in *V. radiata* while 0.450% w/v sample was best for growth in *T. aestivum*. It is important to consider these effects before removing them in sewage water treatment plants.

RESULTS

The growth medium consisted of distilled water and four different concentrations of contraceptive pills containing ethinyl estradiol and norgestrel. For 6 days, 20 seeds were exposed to these solutions in petri-dishes in a lab with a single non-LED yellow light source directly above (**Figure 1**). The number of germinated seeds was recorded on Day 1. On Day 2, the number of *V. radiata* seedlings with visible embryonic leaves and number of *T. aestivum* seedlings with at least two adventitious roots were recorded. Shoot and root lengths were measured using strings and ruler on Day 6. On Day 7, chlorophyll were extracted, centrifuged and analyzed in a spectrophotometer. Absorbance values were used in Arnon's equations to determine the exact chlorophyll a and b concentrations. The procedure to determine chlorophyll concentrations was repeated with five extracts from each solution to attain a mean value.

Figure 1: Experimental Setup of *V. radiata.*

Qualitatively, it was observed that the 0.600% w/v sample of *V. radiata* had almost no root growth compared to the other concentrations. These seedlings did not stand upright towards the light source. Growth of very thin layers of mold was also observed in the 0.600% w/v samples of both of the species. The leaf-blades of *T. aestivum* in the 0.450% w/v sample were greener in colour when compared to the other two concentrations. The mung bean sprouts in the 0.150% w/v sample seemed healthier than the rest; the leaves were slightly larger, and the stems were considerably thicker.

The percentage of seeds germinated varied across the five treatment conditions (**Figure 2**). 95% of *V. radiata* seeds and 70% of *T. aestivum* seeds germinated in the 0.150% and 0.450% w/v samples respectively. These were the highest percentages of germination out of the 20 seeds that were initially set up. The lowest percentage of germinated seeds were in 0.600% w/v of *V. radiata* at 55% and in 0.150% w/v

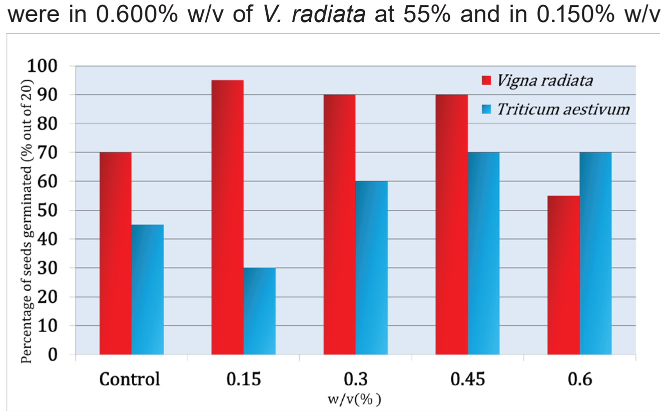

Figure 2: Percentage germination of *V. radiata* and *T. aestivum* on Day 1. We counted the number of seedlings that germinated on Day 1 in each and converted them to percentages out of the initial number of 20 seeds.

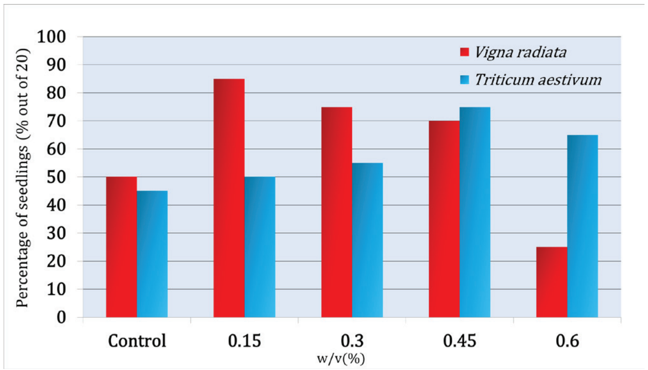

Figure 3: Embryonic leaf proliferation of *V. radiata* and adventitious root proliferation of *T. aestivum* on Day 2. The number of *V. radiata* seedlings with embryonic leaves and the number of *T. aestivum* seedlings that had at least two adventitious roots was counted for each of the different concentrations. This percentage was considered out of the number of seedlings that were germinated on Day 2.

of *T. aestivum* at 30%. The control groups exhibited 70% and 45% germination respectively.

Embryonic leaves sprouted the most in the 0.150% w/v sample of *V. radiata* (85%) and the least from the 0.600% w/v sample (25%) on Day 2 (**Figure 3**). Only 50% of the seeds had the embryonic leaves in the control group. Concentrations of 0.150%, 0.300% and 0.450% w/v exhibited higher leaf proliferation than the control group, but 0.600% w/v concentration exhibited a lower proliferation. The highest percentage of seedlings with two adventitious roots in *T. aestivum* was from the 0.450% w/v sample (75%) and the least was from the control group, which was 45% (**Figure 3**). All concentrations showed higher root tissue proliferation than the control, but after the peak at 0.450% w/v, root proliferation decreased in 0.600% w/v condition.

Highest mean shoot length of *V. radiata* was observed in 0.150% w/v to be 13.81 cm and the lowest mean in 0.600% w/v to be 4.09 cm (**Figure 4**). This was 2.89 cm (26.47%) higher and 6.83 cm (62.55%) lower than the control (10.92 cm). Other than the 0.150% w/v sample, only the 0.300% w/v sample exhibited a marginal increase of mean shoot length from the control, while the other concentrations exhibited lower means. These results were statistically significantly different ($p<0.05$, one-way ANOVA, f-ratio value of 21.30, greater than f-critical value of 2.48).

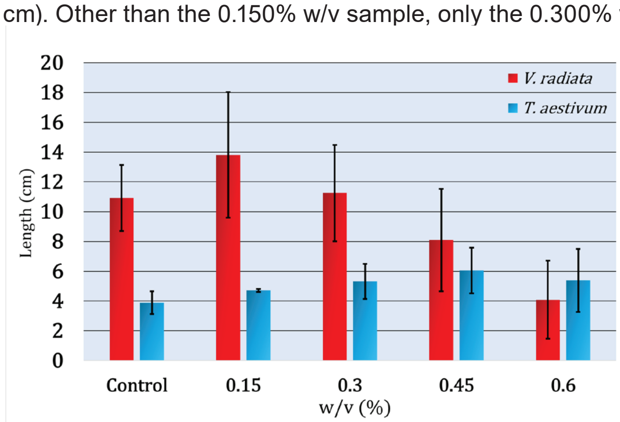

Figure 4: Shoot lengths of *V. radiata* and *T. aestivum* on Day 6. The lengths of shoots of each seedling were measured using strings and rulers. The mean (n=20) was calculated for each concentration and plotted. One-way ANOVA test was conducted to determine statistically significant (*V. radiata*: $p<0.00001$ and *T. aestivum*: $p<0.001321$) differences between the five treatment conditions.

Highest mean shoot length of *T. aestivum* was observed in the 0.450% w/v sample (6.05 cm) and the lowest in the control (3.89 cm) (**Figure 4**). There was an increase of 2.16 cm (55.53%) from the control. These results were statistically significantly different ($p<0.05$, one-way ANOVA, f-ratio value of 5.01, greater than f-critical value of 2.51).

Mean root length was the highest in 0.150% w/v sample (4.77 cm) while the lowest was in the 0.600% w/v sample (1.3 cm) for *V. radiata* (**Figure 5**). This showed an increase of 0.11 cm (2.36%) and a decrease of 3.36 cm (72.10%) from the mean length of the control (4.66 cm). The other three concentrations showed decreased lengths of root from the control. These results were statistically significantly different ($p<0.05$, one-way ANOVA, f-ratio value of 13.92, greater than f-critical value of 2.48).

T. aestivum had the highest mean root length in 0.450% w/v sample at 2.13 cm and the lowest in the control at 1.00 cm (**Figure 5**). An increase of 1.13 cm (113.00%) from the control was observed. All concentrations of sample solution increased the length of root of *T. aestivum* compared to the control. The control had a wider variety of mean root lengths per seedling than the others. These results were statistically significantly different ($p<0.05$, one-way ANOVA, f-ratio value of 7.66, greater than f-critical value of 2.51).

Chlorophyll concentrations fluctuated across the five different conditions for both species of crop-plants. However, highest total chlorophyll concentrations were observed in the 0.150% w/v sample as 10.45 mg/mL and the 0.600% w/v sample as 10.63 mg/mL concentrations of *V. radiata* and *T. aestivum*, respectively, while lowest was observed in both controls, which were 2.28 mg/mL and 5.13 mg/mL respectively (**Figure 6**). This was an increase of 8.17 mg/mL

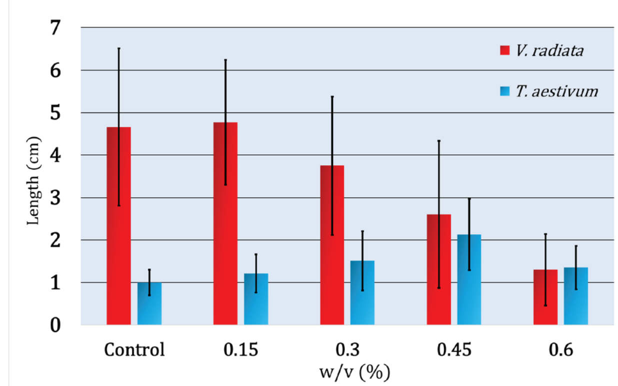

Figure 5: Root lengths of *V. radiata* and *T. aestivum* on Day 6. The lengths of roots of each seedling were measured using strings and rulers. For *T. aestivum*, all the adventitious roots in each seedling were measured, and the mean (n=20) was calculated for every concentration. One-way ANOVA test was conducted to determine statistically significant (*V. radiata*: $p<0.00001$ and *T. aestivum*: $p<0.000036$) differences between the five treatment conditions.

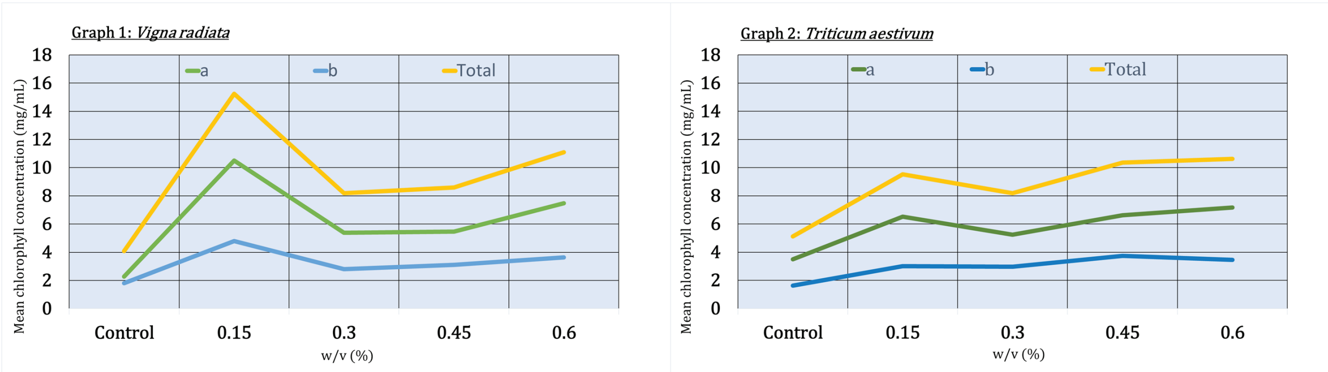

Figure 6: Chlorophyll concentrations *Vigna radiata* and *Triticum aestivum* on Day 6. Mean (n=5) chlorophyll concentrations a, b and total were plotted for both the species. One-way ANOVA test was conducted to determine statistically significant ($p<0.00001$) differences between the five treatment conditions. Standard deviations were far too insignificant to be visible on the graphical representation and were thus omitted.

(358.33%) and 5.5 mg/mL (107.21%) from the control groups. It should also be noted that the second highest chlorophyll concentration was found in the 0.450% w/v sample in *T. aestivum* leaves. This was 10.36 mg/mL, a decrease of only 0.27 mg/mL from the highest. Even though a correlation was difficult to identify between chlorophyll concentration and sample solutions, a similar trend was observed in both the species (increased after 0.000%, decreased after 0.150%, increased after 0.450% and continued to increase up to 0.600%). One-way ANOVA tests were carried out for both datasets at $p<0.05$ (f-ratio of *V. radiata* 281195.04 > 2.87, f-ratio of *T. aestivum* 40109.09>2.87), suggesting statistically significant differences between each obtained data.

DISCUSSION

Progestin-estrogenic tablets affected the growth of both *V. radiata* and *T. aestivum* similarly. The 0.150% w/v condition consistently promoted maximum morphological growth in *V. radiata* while 0.600% w/v condition consistently inhibited it the most. Similarly, *T. aestivum* had maximum morphological growth in the 0.450% w/v sample and the lowest in the control group, except percent germination, which was the lowest in the 0.150% w/v sample.

Variation of chlorophyll concentration was ambiguous, although the highest total chlorophyll concentration was found in the 0.150% w/v sample in *V. radiata*. In *T. aestivum*, the higher concentrations were found in the 0.450% and 0.600% w/v conditions. The fluctuating nature of both the graphs portraying chlorophyll concentrations of the two plants does suggest that a more extensive study of the effects of progestin-estrogenic tablets on chlorophyll concentration is essential to draw a more reliable conclusion. However, the similar pattern in which they vary suggests that the exposure of varied concentrations of progestin-estrogenic contraceptive tablets could have similar effects on plants regardless of the species and the number of cotyledons present in the seeds.

Studies on phytoestrogens and phytoprogestins are not very common. However, one study on the former did suggest their involvement in the initiation of hypersensitive cell death and defense competency in soybean, which leads us to consider its possible effects on the growth of these crops (82). Both Graham's study and Turner *et al.* (2007) suggests some overlap between plant and animal nuclear receptor or other steroidal hormone signaling pathways (82, 30). Phytoestrogens also differ in their binding affinities to different estrogen receptors and modulate the efficacy of receptor binding to the estrogen response element (83), which is a short DNA sequence within the promoter of a gene that regulates transcription (84). Hence, it may be reasonable to speculate that certain concentrations of these hormones improve cell proliferation while other concentrations, perhaps when in excess, diminish cell proliferation by prohibiting enzymatic actions.

Chlorophyll b concentration of *T. aestivum* in the 0.600% w/v sample decreased compared to the 0.450% w/v sample which is unlikely because chlorophyll a concentration increased. Either of these data points may be anomalous. Maximum morphological growth and chlorophyll concentration was consistently found in the 0.150% and 0.450% w/v samples for *V. radiata* and *T. aestivum*, respectively. The only inconsistent result could be the maximum chlorophyll concentration found in the 0.600% w/v sample of *T. aestivum*.

This experimental design could be modified to be carried out in pots of soil instead of petri-dishes, which would be a more accurate model of the actual environmental conditions. Now that we are aware of the potential effects, possibly at a magnified scale, a concentration range in the scale of ng/L or µg/L could be considered in future studies, as it may be more representative of the natural concentration levels. Only 20 seeds were used for each concentration, which could be expanded in the future to broaden the impact of the conclusions. Crop-plants even within the same species can be incredibly varied in terms of growth. A larger sample size would therefore be more representative of the target population, provide stronger results from statistical analysis, and help overcome random errors, assuming that no systematic error is prevalent within the design of the experiment. Additionally, a wider range of concentrations with smaller intervals could be considered for more accuracy and reliability. Incubators should have been used to better control the environmental conditions during the experiment.

Furthermore, this experiment lacked a negative control. Contraceptive pills contain excipient (and inactive) substances like cornstarch and lactose and bulking agents. It is possible that these may have influenced the growth of the seedlings, and it is not clear if the observed effects are solely due to the ethinyl estradiol and norgestrel present in the tablets. But this negative control could not be included as the specific brand of contraceptives used do not contain inert tablets, although many other brands do. The used tablets are monophasic; all tablets contain equal amounts of active and inactive substances and are sold in packs of 21. Future studies should consider using a 28-days pill package so that the inert tablets could be utilized in designing the negative control.

The findings of this study imply that the prevalence of progestin-estrogenic tablets may alter optimum conditions required for cultivation of crops, while at some concentrations the growth may in fact be enhanced. Hence, appropriate regulation of the concentrations of progestins and estrogen may be necessary for sustainable agriculture. One important step in regulating the chemicals could be the improvements of technology in STPs to detect and remove EDCs in the long term. Since installation of such STPs could be very expensive and not inefficient currently (85), sewage pathways should be planned so that only specific streams and rivers are dedicated for disposal of EDCs and installed with STPs, enabling the rest of the water bodies to be safer. Therefore, another implication of this study could be the urgent development of accessible sensors so that EDCs including ethinyl estradiol and norgestrel can be studied more extensively.

If future studies confirm that steroid hormones improve crop growth, then farmers may be able to easily detect and utilize these chemicals available in river-waters to their advantage. This experiment was done over only 6 days and it could be interesting to observe the effects over a longer period, such as 12 weeks, or even the full lifecycle of the crops. One important question that arises is whether traces of these hormones could be found in the wheat and mung bean harvests if they are used as growth-promoters, and could be considered for further studies.

Ethinyl estradiol and norgestrel could potentially also be used as fertilizers directly for irrigation if regulated appropriately. However, the effects of these two chemicals when ingested in humans must be considered as well. Similar studies that delve into the combined effects as well as the individual effects of common EDCs can be pivotal in accurately predicting the fate and effects of these chemicals in the environment. Furthermore, these studies must be done on various other crop species and types to better understand the wide array of effects these could have as suggested by the comparison between the effects on *V. radiata* and *T. aestivum*.

METHODS

The procedure was primarily based on Bowlin (2014) (86), with alterations made to match the availability of equipment and the scope of this study.

Preparation of Stock Solutions of Progestin-estrogenic Tablets

Contraceptive pills were powdered using a mortar and pestle. 0.300±0.001 g weighed using an electronic balance of powder was mixed in 200±0.5 mL of distilled water to make a 0.150% sample solution. Similar method was followed using 0.600 g, 0.900 g and 1.20 g of powdered tablets and 200 mL of distilled water to make 0.300%, 0.450% and 0.600% w/v solutions. Every tablet weighed 0.067 g and contained 0.050 mg ethinyl estradiol and 0.500 mg norgestrel. These were stored at room temperature for use over the duration of the experiment.

Setting-up Seeds with the Apparatus (Figure 1)

A filter paper was kept on the base of a petri-dish. 20 seeds (taken from the same vendor at a local market) of both the species were chosen and rinsed in a 10% bleaching powder solution (10 g in 100 mL of distilled water) for 10-15 minutes. These were spread over the filter paper in each petri-dish. There were 5 petri-dishes for both species, each with 20 seeds, for every concentration of sample solution. All petri-dishes were placed around a hanging non-LED light source, which was turned on every 12 hours throughout the experiment. 25±0.5 mL of sample solution was poured into each petri-dish using 50 mL measuring cylinder every 48 hours for 6 days. Volume of solution in the petri dish did not remain constant throughout the 6 days as it was being used up by the seedlings while some of it also evaporated due to the heat from the non-LED light.

Recording Morphological Growth

Percentage germination i.e. number of seeds germinated, was recorded for all the set-ups (out of 20) on Day 1. A seed was considered to be germinated if the coleoptile had emerged from the seed. On Day 2, the numbers of *V. radiata* seedlings whose embryonic leaves had sprout and the numbers of *T. aestivum* seedlings that had two adventitious roots were counted. Steps 4 to 6 were repeated for all germinated seedlings on Day 3 and Day 6. A string was used to trace the length of the shoot from the bottom of the shoot up to the tip of the apical meristem of *V. radiata* and up to the tip of the leaf blade of *T. aestivum*. The string was then cut at the tips to be measured on a ruler for its length. The same method was repeated for the root length. Strings were used to measure the length from the origin of the primary root of *V. radiata* up to the tip. For *T. aestivum*, the lengths of each adventitious root were measured and the total root lengths per seedling were found.

Determining Chlorophyll Concentration

On Day 6, 2 mg of leaves were measured from each condition of each species using a digital balance. The leaves were pat dried on tissue papers and weighed using a digital balance. The leaves were taken in the mortar and to it 4 mL of 80% acetone was added and crushed with the pestle to soften the cellulose. The crushed suspension was transferred into separate centrifuge tubes. Another 8 mL of acetone was added to make the volume up to 13 mL in each tube and centrifuged at 3500 RPM for 5 minutes. The supernatant for each set of sample solution (0.000%, 0.150%, 0.300%, and 0.600%) was decanted into test tubes. The spectrophotometer was calibrated to zero using 80% acetone as a blank. The absorbance of the supernatant from each sample solution was recorded at wavelengths of 645 nm and 663 nm. Chlorophyll a and b and total chlorophyll content (mg/mL) were determined using the Arnon's equations:

$$\text{Chlorophyll a: } 12.7A_{663} - 2.69A_{645}$$

$$\text{Chlorophyll b: } 22.9\,A_{645} - 4.68\,A_{663}$$

Total Chlorophyll: Chlorophyll a + Chlorophyll b

…where A_{663} and A_{645} are absorbances at 663 nm and 645 nm.

Sample calculations:

Total chlorophyll concentration of *V. radiata* (at 0.150%):

Chlorophyll a:

$$12.7A_{663} - 2.69A_{645} = 12.7(0.906) - 2.69(0.395)$$
$$= 10.44 \text{ mg/mL}$$

Chlorophyll b:

$$22.9\,A_{645} - 4.68\,A_{663} = 22.9(0.395) - 4.68(0.906)$$
$$= 4.81 \text{ mg/mL}$$

Total Chlorophyll:

Chlorophyll a + Chlorophyll b = 10.44 + 4.81 = 15.25 mg/mL

Statistical Analysis

One-way ANOVA tests were done using Microsoft Excel to determine the significance of variance between the mean root and shoot lengths and chlorophyll concentration of five independent groups. There is only one independent variable in an ordered range (0.150% to 0.600%). F-ratio values were greater than the respective f-critical values when $p<0.05$. Significant differences in mean lengths of root and shoot and mean total chlorophyll concentrations were observed. The null hypotheses were thus rejected and the experimental hypotheses stating that, root length, shoot length and total chlorophyll concentrations are affected by exposure to varied concentrations of progestin-estrogenic tablet solution, were accepted.

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REFERENCES

1. Batt, Angela L., *et al.* "Evaluating the Vulnerability of Surface Waters to Antibiotic Contamination from Varying Wastewater Treatment Plant Discharges." *Environmental Pollution*, vol. 142, no. 2, 2006, pp. 295–302., doi:10.1016/j.envpol.2005.10.010.
2. Gaw, Sally, *et al.* "Sources, Impacts and Trends of Pharmaceuticals in the Marine and Coastal Environment." *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 369, no. 1656, 2014, p. 20130572., doi:10.1098/rstb.2013.0572.
3. Glassmeyer, Susan T., *et al.* "Transport of Chemical and Microbial Compounds from Known Wastewater Discharges: Potential for Use as Indicators of Human Fecal Contamination." *Environmental Science & Technology*, vol. 39, no. 14, 2005, pp. 5157–5169., doi:10.1021/es048120k.
4. Hummel, Daniela, *et al.* "Simultaneous Determination of Psychoactive Drugs and Their Metabolites in Aqueous Matrices by Liquid Chromatography Mass Spectrometry†." *Environmental Science & Technology*, vol. 40, no. 23, 2006, pp. 7321–7328., doi:10.1021/es061740w.
5. Pharmaceuticals in Drinking Water. WHO Press, https://www.who.int/water_sanitation_health/publications/2011/pharmaceuticals_20110601.pdf.
6. Oaks, J. Lindsay, *et al.* "Diclofenac Residues as the Cause of Vulture Population Decline in Pakistan." *Nature*, vol. 427, no. 6975, 2004, pp. 630–633., doi:10.1038/nature02317.
7. Crisp, Thomas M., *et al.* Special Report on Environmental Endocrine Disruption: An Effects Assessment Analysis. *USEPA*, https://archive.epa.gov/raf/web/pdf/endocrine.pdf.
8. Ingerslev, Flemming, and Bent Halling-Sørensen. Evaluation of Analytical Chemical Methods for Detection of Estrogens in the Environment. Danish Environmental Protection Agency, 2003, https://pdfs.semanticscholar.org/4bdb/226151b93f236f3bbff22bb87823c070c9df.pdf?_ga=2.133698126.1516064087.1581290267-64757373.1581290267.
9. Kinnberg, Karin. Evaluation of in Vitro Assays for Determination of Estrogenic Activity in the Environment. Danish Environmental Protection Agency, 2003, https://pdfs.semanticscholar.org/13e2/907c526739a46d2c9fd9442a3835dd9c1536.pdf?_ga=2.134027086.1516064087.1581290267-64757373.1581290267.
10. Bradley, Paul M., *et al.* "Biodegradation of 17β-Estradiol, Estrone and Testosterone in Stream Sediments." *Environmental Science & Technology*, vol. 43, no. 6, 2009, pp. 1902–1910., doi:10.1021/es802797j.
11. Fernandez, Marc P., *et al.* "An Assessment of Estrogenic Organic Contaminants in Canadian Wastewaters." *Science of The Total Environment*, vol. 373, no. 1, 2007, pp. 250–269., doi:10.1016/j.scitotenv.2006.11.018.
12. Fick, Jerker, *et al.* "Therapeutic Levels of Levonorgestrel Detected in Blood Plasma of Fish: Results from Screening

Rainbow Trout Exposed to Treated Sewage Effluents." *Environmental Science & Technology*, vol. 44, no. 7, 2010, pp. 2661–2666., doi:10.1021/es903440m.

13. Hutchins, Stephen R., *et al.* "Analysis of Lagoon Samples from Different Concentrated Animal Feeding Operations for Estrogens and Estrogen Conjugates." *Environmental Science & Technology*, vol. 41, no. 3, 2007, pp. 738–744., doi:10.1021/es062234.
14. Duckett, S. K., and J. G. Andrae. "Implant Strategies in an Integrated Beef Production System." *Journal of Animal Science*, vol. 79, no. E-Suppl, 2001, doi:10.2527/jas2001.79e-supple110x.
15. Preston, R.I. "Hormone Containing Growth Promoting Implants in Farmed Livestock." *Advanced Drug Delivery Reviews*, vol. 38, no. 2, 1999, pp. 123–138., doi:10.1016/s0169-409x(99)00012-5.
16. Bartelt-Hunt, Shannon L., *et al.* "Effect of Growth Promotants on the Occurrence of Endogenous and Synthetic Steroid Hormones on Feedlot Soils and in Runoff from Beef Cattle Feeding Operations." *Environmental Science & Technology*, vol. 46, no. 3, 2012, pp. 1352–1360., doi:10.1021/es202680q.
17. Blair, R. M. "The Estrogen Receptor Relative Binding Affinities of 188 Natural and Xenochemicals: Structural Diversity of Ligands." *Toxicological Sciences*, vol. 54, no. 1, Jan. 2000, pp. 138–153., doi:10.1093/toxsci/54.1.138.
18. Bauer, E. R. S., *et al.* "Characterisation of the Affinity of Different Anabolics and Synthetic Hormones to the Human Androgen Receptor, Human Sex Hormone Binding Globulin and to the Bovine Progestin Receptor." *Apmis*, vol. 108, no. 12, 2000, pp. 838–846., doi:10.1111/j.1600-0463.2000.tb00007.x.
19. Combined Estrogen-Progestogen Contraceptives and Combined Estrogen-Progestogen Menopausal Therapy. *IARC*, 2007, https://www.ncbi.nlm.nih.gov/books/NBK321672/.
20. "NCI Thesaurus." National Institutes of Health, U.S. Department of Health and Human Services, ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI Thesaurus&code=C703.
21. Regal, Patricia, *et al.* "Natural Hormones in Food-Producing Animals:Legal Measurements and Analytical Implications." *Food Production - Approaches, Challenges and Tasks*, 2012, doi:10.5772/26789.
22. Schiffer, B, *et al.* "The Fate of Trenbolone Acetate and Melengestrol Acetate after Application as Growth Promoters in Cattle: Environmental Studies." *Environmental Health Perspectives*, vol. 109, no. 11, 2001, pp. 1145–1151., doi:10.1289/ehp.011091145.
23. Adeel, Muhammad, *et al.* "Environmental Impact of Estrogens on Human, Animal and Plant Life: A Critical Review." *Environment International*, vol. 99, 2017, pp. 107–119., doi:10.1016/j.envint.2016.12.010.
24. Durhan, Elizabeth J., *et al.* "Identification of Metabolites of Trenbolone Acetate in Androgenic Runoff from a Beef Feedlot." *Environmental Health Perspectives*, vol. 114, no. Suppl 1, 2006, pp. 65–68., doi:10.1289/ehp.8055.
25. Orlando, Edward F, *et al.* "Endocrine-Disrupting Effects of Cattle Feedlot Effluent on an Aquatic Sentinel Species, the Fathead Minnow." *Environmental Health Perspectives*, vol. 112, no. 3, 2004, pp. 353–358., doi:10.1289/ehp.6591.
26. Koh, Y.k.k., *et al.* "Treatment And Removal Strategies For Estrogens From Wastewater." *Environmental Technology*, vol. 29, no. 3, 2008, pp. 245–267., doi:10.1080/09593330802099122.
27. Snyder, S., *et al.* "Analytical Methods Used to Measure Endocrine Disrupting Compounds in Water." *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, vol. 7, no. 4, 2003, pp. 224–234., doi:10.1061/(asce)1090-025x(2003)7:4(224).
28. "NCI Thesaurus." National Institutes of Health, U.S. Department of Health and Human Services, ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI_Thesaurus&ns=NCI_Thesaurus&code=C486.
29. Kuhl, H. "Pharmacology of Estrogens and Progestogens: Influence of Different Routes of Administration." *Climacteric*, vol. 8, no. sup1, 2005, pp. 3–63., doi:10.1080/13697130500148875.
30. Turner, Joseph V., *et al.* "Molecular Aspects of Phytoestrogen Selective Binding at Estrogen Receptors." *Journal of Pharmaceutical Sciences*, vol. 96, no. 8, 2007, pp. 1879–1885., doi:10.1002/jps.20987.
31. Coss, Christopher C., *et al.* "Preclinical Characterization of a Novel Diphenyl Benzamide Selective ERα Agonist for Hormone Therapy in Prostate Cancer." *Endocrinology*, vol. 153, no. 3, 2012, pp. 1070–1081., doi:10.1210/en.2011-1608.
32. Mp, Ekback. "Hirsutism, What to Do?" International *Journal of Endocrinology and Metabolic Disorders*, vol. 3, no. 3, 2017, doi:10.16966/2380-548x.140.
33. Nieschlag, E., *et al.* Testosterone: Action, Deficiency, Substitution. *Cambridge University Press*, 2012.
34. Christiansen, Lisette Bachmann, *et al.* The Effect of Estrogenic Compounds and Their Fate in Sewage Treatment Plants and Nature. Danish Environmental Protection Agency, https://www.yumpu.com/en/document/read/14087592/feminisation-of-fish-miljstyrelsen.
35. Jobling, Susan, *et al.* "Widespread Sexual Disruption in Wild Fish." *Environmental Science & Technology*, vol. 32, no. 17, 1998, pp. 2498–2506., doi:10.1021/es9710870.
36. Bhandari, Ramji K., *et al.* "Transgenerational Effects from Early Developmental Exposures to Bisphenol A or 17α-Ethinylestradiol in Medaka, Oryzias Latipes." *Scientific Reports*, vol. 5, no. 1, 2015, doi:10.1038/srep09303. 37.
37. Wang, Bonnie, *et al.* "The involvement of CYP3A4 and CYP2C9 in the metabolism of 17 alpha-ethinyl estradiol." *Drug Metabolism and Disposition*, vol. 32, no. 11, Oct.

2004, pp. 1209–1212., doi:10.1124/dmd.104.000182.
38. Caldwell, Daniel J., *et al.* "Predicted-No-Effect Concentrations for the Steroid Estrogens Estrone, 17β-Estradiol, Estriol, and 17α-Ethinylestradiol." *Environmental Toxicology and Chemistry*, vol. 31, no. 6, 27 Apr. 2012, pp. 1396–1406., doi:10.1002/etc.1825.
39. Baronti, Chiara, *et al.* "Monitoring Natural and Synthetic Estrogens at Activated Sludge Sewage Treatment Plants and in a Receiving River Water." *Environmental Science & Technology*, vol. 34, no. 24, Dec. 2000, pp. 5059–5066., doi:10.1021/es001359q.
40. Ifelebuegu, A. O. "Removal of Endocrine Disrupting Chemicals in Wastewater Treatment Applications." Coventry University, 2013.
41. Evans, Ginger, and Eliza L. Sutton. "Oral Contraception." *Medical Clinics of North America*, vol. 99, no. 3, 2015, pp. 479–503., doi:10.1016/j.mcna.2015.01.004.
42. Sitruk-Ware, Regine, and Anita Nath. "The Use of Newer Progestins for Contraception." *Contraception*, vol. 82, no. 5, 2010, pp. 410–417., doi:10.1016/j.contraception.2010.04.004.
43. Ellestad, Laura E., *et al.* "Environmental Gestagens Activate Fathead Minnow (Pimephales Promelas) Nuclear Progesterone and Androgen Receptors in Vitro." *Environmental Science & Technology*, vol. 48, no. 14, 2014, pp. 8179–8187., doi:10.1021/es501428u.
44. Schoonen, W.g.e.j, *et al.* "Contraceptive Progestins. Various 11-Substituents Combined with Four 17-Substituents: 17α-Ethynyl, Five- and Six-Membered Spiromethylene Ethers or Six-Membered Spiromethylene Lactones." *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 74, no. 3, 2000, pp. 109–123., doi:10.1016/s0960-0760(00)00094-7.
45. García-Becerra Rocio, *et al.* "The Intrinsic Transcriptional Estrogenic Activity of a Non-Phenolic Derivative of Levonorgestrel Is Mediated via the Estrogen Receptor-α." *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 82, no. 4-5, 2002, pp. 333–341., doi:10.1016/s0960-0760(02)00192-9.
46. Runnalls, Tamsin J., *et al.* "Several Synthetic Progestins with Different Potencies Adversely Affect Reproduction of Fish." *Environmental Science & Technology*, vol. 47, no. 4, Aug. 2013, pp. 2077–2084., doi:10.1021/es3048834.
47. Zucchi, Sara, *et al.* "Progestins and Antiprogestins Affect Gene Expression in Early Development in Zebrafish (Danio Rerio) at Environmental Concentrations." *Environmental Science & Technology*, vol. 46, no. 9, 2012, pp. 5183–5192., doi:10.1021/es300231y.
48. Zeilinger, Jana, *et al.* "Effects Of Synthetic Gestagens On Fish Reproduction." *Environmental Toxicology and Chemistry*, vol. 28, no. 12, 2009, p. 2663-2670., doi:10.1897/08-485.1.
49. Fent, Karl. "Progestins as Endocrine Disrupters in Aquatic Ecosystems: Concentrations, Effects and Risk Assessment." *Environment International*, vol. 84, 2015, pp. 115–130., doi:10.1016/j.envint.2015.06.012.
50. Jenkins, R. L. "Androstenedione and Progesterone in the Sediment of a River Receiving Paper Mill Effluent." *Toxicological Sciences*, vol. 73, no. 1, 2003, pp. 53–59., doi:10.1093/toxsci/kfg042.
51. Liu, Shan, *et al.* "Steroids in Marine Aquaculture Farms Surrounding Hailing Island, South China: Occurrence, Bioconcentration, and Human Dietary Exposure." *Science of The Total Environment*, vol. 502, 2015a, pp. 400–407., doi:10.1016/j.scitotenv.2014.09.039.
52. J.K., Amory. "Testosterone/Progestin Regimens: a Realistic Option for Male Contraception?" *Current Opinion in Investigational Drugs*, vol. 5, no. 10, 2004, pp. 1025–1030., https://www.ncbi.nlm.nih.gov/pubmed/15535423.
53. Craig, Charles R., and Robert E. Stitzel. Modern Pharmacology with Clinical Applications. *Lippincott*, 2004.
54. "Estrogen and Progestin (Oral Contraceptives): MedlinePlus Drug Information." MedlinePlus, U.S. National Library of Medicine, medlineplus.gov/druginfo/meds/a601050.html.
55. Dodgen, Laurel K., *et al.* "Effect of Transpiration on Plant Accumulation and Translocation of PPCP/EDCs." *Environmental Pollution*, vol. 198, 2015, pp. 144–153., doi:10.1016/j.envpol.2015.01.002.
56. Iino, Mayumi, *et al.* "Progesterone: Its Occurrence in Plants and Involvement in Plant Growth." *Phytochemistry*, vol. 68, no. 12, 2007, pp. 1664–1673., doi:10.1016/j.phytochem.2007.04.002.
57. Janeczko A. Influence of Selected Steroids on Plant Physiological Processes-Especially Flowering Induction. Krakow: Ph.D. Dissertation, Agriculture University, 2000.
58. Janeczko, Anna, and Władysław Filek. "Stimulation of Generative Development in Partly Vernalized Winter Wheat by Animal Sex Hormones." *Acta Physiologiae Plantarum*, vol. 24, no. 3, 2002, pp. 291–295., doi:10.1007/s11738-002-0054-0.
59. Nirmala, G.C., *et al.* "Effect of Estrogen and Progesterone on Seed Germination." *Veterinary World*, vol. 1, no. 8, 2008, pp. 241–242., http://www.veterinaryworld.org/2008/August/Effect%20of%20Estrogen%20and%20Progesterone%20on%20seed%20germination.pdf
60. Dabrosca, Brigida, *et al.* "Phytotoxicity Evaluation of Five Pharmaceutical Pollutants Detected in Surface Water on Germination and Growth of Cultivated and Spontaneous Plants." *Journal of Environmental Science and Health*, Part A, vol. 43, no. 3, 2008, pp. 285–294., doi:10.1080/10934520701792803.
61. Bhattacharya, B., and K. Gupta. "Steroid Hormone Effects on Growth and Apical Dominance of Sunflower." *Phytochemistry*, vol. 20, no. 5, 1981, pp. 989–991., doi:10.1016/0031-9422(81)83014-2.
62. Vaisman, I., *et al.* "Reducing Ground□Water Pollution from Municipal Waste□Water Irrigation of Rhodes Grass Grown on Sand Dunes." *Journal of Environmental*

Quality, vol. 10, no. 4, 1981, pp. 434–439., doi:10.2134/jeq1981.00472425001000040002x.

63. Bielorai, H., *et al.* “Drip Irrigation of Cotton with Treated Municipal Effluents: I. Yield Response.” *Journal of Environmental Quality*, vol. 13, no. 2, 1984, pp. 231–234., doi:10.2134/jeq1984.00472425001300020011x.
64. Shore, Laurence S., *et al.* “Effects of Estrone and 17 Beta-Estradiol on Vegetative Growth of Medicago Sativa.” *Physiologia Plantarum*, vol. 84, no. 2, 1992, pp. 217–222., doi:10.1111/j.1399-3054.1992.tb04656.x.
65. Perron, Marie-Claude, and Philippe Juneau. “Effect of Endocrine Disrupters on Photosystem II Energy Fluxes of Green Algae and Cyanobacteria.” *Environmental Research*, vol. 111, no. 4, 2011, pp. 520–529., doi:10.1016/j.envres.2011.02.013.
66. Upadhyay, Pallavi, and Camelia Maier. “Effects of 17β-Estradiol on Growth, Primary Metabolism, Phenylpropanoid-Flavonoid Pathways and Pathogen Resistance in Arabidopsis Thaliana” *American Journal of Plant Sciences*, vol. 07, no. 13, 2016, pp. 1693–1710., doi:10.4236/ajps.2016.713160.
67. Ylstra, B., *et al.* “Steroid Hormones Stimulate Germination and Tube Growth of in Vitro Matured Tobacco Pollen.” *Plant Physiology*, vol. 107, no. 2, Jan. 1995, pp. 639–643., doi:10.1104/pp.107.2.639.
68. Erdal, Serkan, and Rahmi Dumlupinar. “Progesterone and β-Estradiol Stimulate Seed Germination in Chickpea by Causing Important Changes in Biochemical Parameters.” *Zeitschrift Für Naturforschung C*, vol. 65, no. 3-4, Jan. 2010, pp. 239–244., doi:10.1515/znc-2010-3-412.
69. Anwar, F., *et al.* “Chemical Composition and Antioxidant Activity of Seeds of Different Cultivars of Mungbean.” *Journal of Food Science*, vol. 72, no. 7, 2007, doi:10.1111/j.1750-3841.2007.00462.x.
70. Kudre, Tanaji G, *et al.* “Comparative Study on Chemical Compositions and Properties of Protein Isolates from Mung Bean, Black Bean and Bambara Groundnut.” *Journal of the Science of Food and Agriculture*, vol. 93, no. 10, Dec. 2013, pp. 2429–2436., doi:10.1002/jsfa.6052..
71. Meelu, O.P. *et al.* “Grain Yield Responses in Rice to Eight Tropical Green Manures.” *Tropical Agriculture*, vol. 69, no. 2, 1992, pp. 133–136.
72. Devendra, C., *et al.* “Food-Feed Systems in Asia - Review -.” Asian-Australasian *Journal of Animal Science*s, vol. 14, no. 5, Jan. 2001, pp. 733–745., doi:10.5713/ajas.2001.733.
73. Sustainable Crop Rotations with Cover Crops. The Ohio State University, 2009, https://pdfs.semanticscholar.org/bd8a/0a36820baf18110e83835117a4545cdcd3d2.pdf?_ga=2.173349731.1516064087.1581290267-64757373.1581290267.
74. Tang, Dongyan, *et al.* “A Review of Phytochemistry, Metabolite Changes, and Medicinal Uses of the Common Food Mung Bean and Its Sprouts (*Vigna radiata*).” *Chemistry Central Journa*l, vol. 8, no. 1, 2014, doi:10.1186/1752-153x-8-4.
75. United Nations, Food and Agriculture Organization. World food situation: FAO cereal supply and demand brief. Rome, Italy, 2016.
76. United Nations, Food and Agriculture Organization, Statistics Division. "Crops/World Total/Wheat/Production Quantity/2014 (pick list)." 2015.
77. “Wheat.” FoodData Central, US Department of Agriculture, fdc.nal.usda.gov/fdc-app.html#/food-details/339623/nutrients.
78. Day, L., *et al.* “Wheat-Gluten Uses and Industry Needs.” *Trends in Food Science & Technology*, vol. 17, no. 2, 2006, pp. 82–90., doi:10.1016/j.tifs.2005.10.003.
79. Davis, Tim D., and Bruce E. Haissig. Biology of Adventitious Root Formation. Plenum, 1994, https://link.springer.com/content/pdf/10.1007/978-1-4757-9492-2.pdf.
80. Wise, Amber, *et al.* “Are Oral Contraceptives a Significant Contributor to the Estrogenicity of Drinking Water?.” *Environmental Science & Technology*, vol. 45, no. 1, 2011, pp. 51–60., doi:10.1021/es1014482.
81. Fleischer, Walter E. “The Relation Between Chlorophyll Content And Rate Of Photosynthesis.” *The Journal of General Physiology*, vol. 18, no. 4, 1935, pp. 573–597., doi:10.1085/jgp.18.4.573.
82. Graham, T.L. “Biosynthesis and Distribution of Phytoestrogens and Their Roles in Plant Defense, Signal Transduction, and Cell-to-Cell Signaling.” *Journal of Medicinal Food*, vol. 2, no. 3-4, 1999, pp. 93–97., doi:10.1089/jmf.1999.2.93.
83. Kostelac, Drazen, *et al.* “Phytoestrogens Modulate Binding Response of Estrogen Receptors α and β to the Estrogen Response Element.” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 26, 2003, pp. 7632–7635., doi:10.1021/jf034427b.
84. Gruber, Christian J, *et al.* “Anatomy of the Estrogen Response Element.” Trends in *Endocrinology & Metabolism*, vol. 15, no. 2, 2004, pp. 73–78., doi:10.1016/j.tem.2004.01.008.
85. Liu, Ze-Hua, *et al.* “Removal Mechanisms for Endocrine Disrupting Compounds (EDCs) in Wastewater Treatment — Physical Means, Biodegradation, and Chemical Advanced Oxidation: A Review.” *Science of The Total Environment*, vol. 407, no. 2, 2009, pp. 731–748., doi:10.1016/j.scitotenv.2008.08.039.
86. Bowlin, Kelsey Marie. "Effects of β-Estradiol on the Germination and Growth in Zea Mays." Northwest Missouri State University, 2014, https://www.nwmissouri.edu/library/theses/2014/BowlinKelseyM.pdf