International Conference on Sustainable Biotechnology (ICoSBi)
Available online at: https://sicash.sliit.lk/

Investigating Nicotine's Role in *In vitro* Seed Germination and Seedling Growth of Tomato

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Abstract

Nicotine is an addictive alkaloid found primarily in tobacco plants. Nicotine can be leached out into the soil through discarded tobacco products and can be taken up through the roots of plants and accumulated within them. Tomato (*Solanum lycopersicum*) is one of the major crops in the world that also contributes to the Sri Lankan economy. To study the effect of nicotine on plant growth and development, crude nicotine was extracted from dried tobacco leaves using the solvent extraction method. The study was conducted *in vitro* conditions. Tomato seeds were exposed to 20 µL/L, 50 µL/L, 100 µL/L, and 200 µL/L of crude nicotine (CN) in two types of media (media supplemented with 0.1 mg/L IAA and 2 mg/L BAP, and the other media without hormones). The seed germination rate and the growth and development of the seedlings were observed over a period of 8 weeks. The seed sprouting rate was highest in 100 µL/L CN in the media supplemented with hormones. The seed sprouting rate was highest in 50 µL/L CN in the hormone-free media. In the hormone-supplemented media, CN inhibited the growth of seedlings, especially in the 200 µL/L CN, where even the root growth was severely stunted. In the hormone-free media, some seedlings that were exposed to CN showed nutrient deficiency symptoms, and adventitious roots were observed coming from the leaves of some seedlings. This study provides insight into how nicotine from tobacco and nicotine products can affect seed germination and seedling growth.

Keywords: Crude nicotine, plant tissue culture, seed germination, seedling growth

https://doi.org/10.54389/AUGC8799

Introduction

Nicotine, the primary alkaloid in tobacco, has both stimulating and tranquilizing effects. Its addictive nature has fuelled the resurgence of nicotine products, including cigarettes, vapes, snuff, and nicotine replacement therapies like gums and patches. Nicotine poses health risks, impacting immune response and reproductive health. Crude nicotine (CN), a yellow oily liquid with a strong tobacco smell, was discovered in 1571 and primarily consists of nicotine in most tobacco varieties (Shoji et al., 2024).

Nicotine, the primary alkaloid in tobacco, poses environmental risks as it leaches into soil from tobacco and nicotine products. These leachates pollute soil and aquatic ecosystems, disrupting ecological balance. (Cheng et al., 2021). Nicotine stress can affect plants grown in soil previously used for tobacco cultivation, as nicotine released from tobacco plants acts as an allelopathic agent (Selmar et al., 2018). These alkaloids influence seed germination, plant growth, and development, with uptake varying by species. Tomato naturally contains low nicotine levels (2.4–6.0 $\mu g\ kg^{-1}$) but has been shown to absorb nicotine, with studies indicating increased yield in tomato scions grafted onto tobacco stocks (Yasinok et al., 2009).
Cigarette butts are among the most littered items globally, polluting the environment. Along with newer alternatives like vapes, nicotine contamination in soil affects plant growth and development. Interestingly, while tobacco enhances plant growth (Asif Pathan et al., 2018; Green et al., 2023), nicotine products such as e-juices inhibit it (Green et al., 2023). While grafting tomato onto tobacco can impact yield and nicotine content, studies show that discarded cigarette butts reduce germination and plant productivity (Beutel et al., 2021). The effects of tobacco waste vary depending on plant species and nicotine uptake. If nicotine levels exceed the maximum residue limit, nicotine leachate could pose a health risk.

Tomato is a major global crop, with production reaching over 186 million metric tons in 2022 (Ritchie et al., 2023). Sri Lanka contributed 88,081 tons, generating $41,500 in export revenue. The *Thilina* variety, native to Sri Lanka, produces fruit within two months.

With the rise of e-cigarette use and persistent cigarette butt littering, studying nicotine's impact on plant growth and development is long overdue. This study examines its effects on seed germination and seedling growth under *in vitro* conditions, focusing on germination rates, shoot and root growth, and leaf formation in tomato seedlings.

Methodology

Extraction of crude nicotine

Tobacco leaves were sundried and ground until dust was formed. The tobacco dust was sieved through a 60-sieve (2.5 mm) to get a fine powder. The tobacco dust was mixed with a concentrated NaOH solution and stirred well. The solution was filtered through a Whatman I filter paper, and the filtrate was added to a separation funnel, and solvent extraction was carried out using 1:1 volume of diethyl ether. The solvent extraction was repeated, saving the ether phase. The ether phase was dried using anhydrous K_2SO_4. The dried ether phase was added to evaporating dishes, and the diethyl ether was allowed to completely evaporate to get crude nicotine.

In vitro experiment

Tomato seeds of the Thilina variety were grown on full MS medium, either hormone-free or supplemented with 2 mg/L BAP and 0.1 mg/L IAA (Ahmed et al., 2023). Both media were treated with CN at 20, 50, 100, and 200 µL/L. Each CN concentration was tested on 40 seeds (20 per medium type), with 40 additional seeds used as controls.

Surface sterilization of the seeds was performed according to the method described by Ahmed et al. (2023). The seeds were soaked in sterile distilled water for one hour, rinsed, and then treated with 70% isopropyl alcohol for 30 seconds. After rinsing again, they were stirred in a 2% Clorox solution with Tween 20 for 15 minutes. The seeds were rinsed four times in sterile distilled water before being established in the media.

The seeds were assessed for any contamination and for any potential sprouting.

The seeds were allowed to sprout in controlled conditions, with the room temperature at 25 – 30℃ ± 0.5 ℃, light intensity at 3240 – 3780 lux, and the humidity range at 50 – 60 % ± 3 %. The seed germination rate and the seedling growth pattern were observed over a period of 8 weeks.

Data analysis

Data were analysed using the Jamovi 2.3.28 package. A one-way Analysis of Variance (ANOVA) was conducted to evaluate the effect of CN treatment on seed sprouting in hormone-supplemented media. The dataset included three groups: Trials, Nicotine content, and Seed sprouting rate, each with 10 observations.

Results

Statistical analysis

***In vitro* - Hormone-supplemented media**

Table 1 shows the results of one-way ANOVA performed on the seed germination rate for seeds in the *in vitro* hormone-supplemented media. Since $P<0.05$, CN has a significant effect on seed sprouting rate on hormone-supplemented media.

H_0: CN does not have an effect on seed sprouting on hormone-supplemented media.

H_1: CN has an effect on seed sprouting on hormone-supplemented media.

Table 1: *Statistical analysis of seed germination in hormone-supplemented media.*

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	29482.664	2	14741.33	5.487504	0.013151	3.521893
Within Groups	51040.566	19	2686.346			
Total	80523.23	21				

***In vitro* – Hormone-free media**

Table 2 shows the results of one-way ANOVA performed on the seed germination rate for seeds in the in vitro hormone-free media. Since P<0.05, CN has a significant effect on seed sprouting on hormone-free media.

H_0: CN does not have an effect on seed sprouting on hormone-free media.

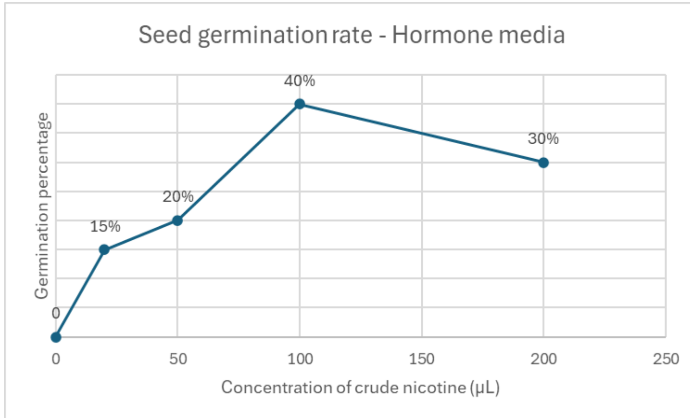
H_1: CN has an effect on seed sprouting on hormone-free media.

Table 2: *The results of one-way ANOVA performed on the seed germination rate for seeds in the in vitro hormone-free media.*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	29590.38764	2	14795.19	5.507557	0.012985	3.521893
Within Groups	51040.536	19	2686.344			
Total	80630.92364	21				

***In vitro* experiment**

The observations were made over a period of 8 weeks. As seen in Figure 1, In hormone-supplemented media, lower CN concentrations inhibited germination, whereas the highest rate was observed at 100 μL/L, declining at 200 μL/L. In hormone-free media, lower CN concentrations enhanced germination, while higher concentrations had an inhibiting effect.



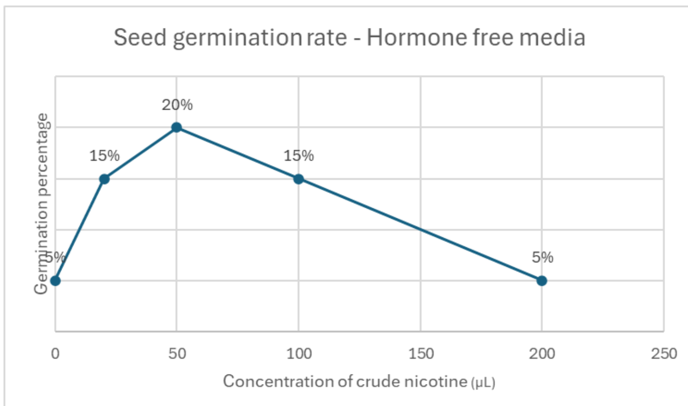


Figure 1: *Chart 1 indicates the germination rate of seeds in the hormone-supplemented media and Chart 2 indicates the germination rate of seeds in the hormone-free media.*

Seedlings in control media exhibited normal growth for the most part, whereas CN-supplemented media resulted in abnormal development. At 50 µL/L CN, normal growth was observed initially, but shoot elongation ceased after two weeks, consistent with results from 100 µL/L CN, where early growth was faster than the control but later stalled.

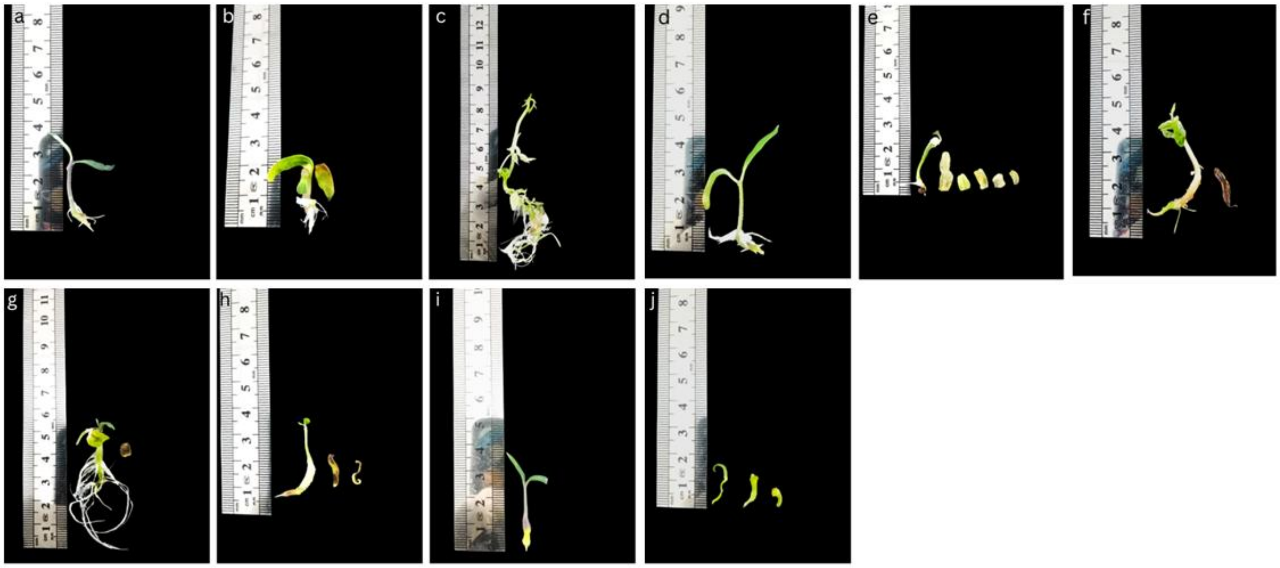


Figure 2: *A-C seedlings grown in the control media (without CN); D- seeding grown in media with 50 µL/L of CN; E-G Seedlings grown in media supplemented with 100 µL/L CN; H-J seedlings grown in media supplemented with 200 µL/L CN. Observations made after 8 weeks.*

CN significantly affected root growth as seen in Figure 2. Higher concentrations (200 µL/L) of CN led to stunted shoots, swollen roots, and minimal lateral roots. Additionally, CN may influence cell differentiation, as seedlings in 200 µL/L CN lacked normal development.

Seedlings in hormone-supplemented media were smaller than those in hormone-free media. Additionally, CN-induced hormones may have antagonistic effects when combined with externally supplemented hormones (auxin and cytokinin), as seedlings of the same CN concentration exhibited different morphologies in the two types of media. Overall, seedlings in hormone-free media were taller, with larger leaves and fewer abnormalities.

In hormone-free media, seedlings supplemented with 20 µL/L and 50 µL/L CN showed normal growth. At 100 µL/L CN, 66.67% maintained normal development, whereas at 200 µL/L CN, all exhibited abnormal growth, confirming that higher CN concentrations increase abnormal seedling development.

Figure 3: *K- the seedling from the control; L- Adventitious roots were observed to be coming from the top of the shoot (in white).; M- Seedling from the 20 μL/L CN media.; N to Q- seedlings from the 50 μL/L CN media; R and S- the leaves of all the seedlings growing in nicotine supplemented media showed were translucent to some degree. R and S are leaves from the seedlings growing in the 50 μL/L CN media.; T to V- Seedlings from the 100 μL/L CN media; W- Seedling from the 200 μL/L media.*

Over time, all CN-treated seedlings showed symptoms of nitrogen deficiency, including yellowing and senescence of older leaves, with some areas appearing translucent. Another observation that was made in the hormone free media, even in the seedlings in the control media, are that adventitious roots were formed in some of the seedlings as seen in figure 3-L.

Discussion

In this study, the effects CN on the in vitro seed germination and growth of seedlings were studied *in vitro.* CN extracted from dried tobacco leaves was applied at varying concentrations to hormone-supplemented and hormone-free media. The germination rate and the effect CN on plant growth were studied.
In hormone-supplemented media, CN inhibited seed germination at lower concentrations, but at higher concentrations, the seed germination rate was the same as the control. However, after germination, lower CN concentrations promoted healthier seedling growth compared to higher concentrations. This aligns partly with findings in broad bean seeds (Cheng et al., 2021), where mild nicotine stress had a hormesis effect, though nicotine also exhibited allelopathic inhibition during germination. These differences may stem from variations in plant morphology and bioprocesses between broad beans and tomatoes. Supplemented growth hormones (Indole-3-acetic acid (IAA) and 6-Benzylaminopurine (BAP)) were studied for their effects on shoot development. The combination of endogenous CN-induced hormones

and externally provided IAA and BAP appeared to inhibit shoot growth, potentially due to the influence of antagonistic hormones like ABA (Abscisic acid). Additionally, CN may alter the levels of other plant hormones such as salicylic acid, jasmonic acid, and IAA, contributing to the observed growth patterns. (Cheng et al., 2021)

Higher CN concentrations impact cell differentiation, as abnormal seedling growth increases with CN levels. At 200 μL/L CN, one seedling exhibited abnormal cell and tissue differentiation. Due to the limited research on nicotine's effects on plant cell differentiation, its precise mechanism remains unclear.

Seedlings in hormone-free test media exhibited yellowing and senescence, with some younger leaves appearing translucent. While the exact cause remains unidentified, nitrogen deficiency is suspected, as enzymes involved in nitrogen metabolism are linked to chlorophyll synthesis. Nitrogen deficiency reduces spongy parenchyma and palisade cell volume, increasing air chambers within the leaf, which eventually leads to senescence (Wen et al., 2019). CN induces nitrogen deficiency, leading to reduced chlorophyll production. Studies on nicotine's effects confirm decreased chlorophyll a, chlorophyll b, carotenoids, and xanthophyll levels in leaves exposed to nicotine, indicating its inhibitory role in chlorophyll synthesis (Asif Pathan et al., 2018).

Nitrogen deficiency symptoms were absent in seedlings grown in media supplemented with BAP and IAA. While BAP does not affect nitrogen-deficient plants, IAA plays a crucial role in nutrient uptake and mobilization. Nitrogen deficiency disrupts mineral absorption and overall nutrient efficiency, triggering reactive oxygen species that damage cellular metabolism. IAA mitigates oxidative stress in nutrient-deficient plants, improving their health under such conditions. (Liu et al., 2024). IAA enhances nitrogen uptake, utilization, and efficiency in plants. However, leaf translucency may also result from natural senescence. The topmost leaves, shielded from direct light by the culture tube caps, may have reduced chlorophyll synthesis, relying on sucrose and nutrients already present in the media. However, nitrogen deficiency remains the likely cause, as control plant leaves showed no such translucency.

Some seedlings, including controls in hormone-free media, developed adventitious roots from leaves and shoots which is an unusual occurrence in tomato plants. If nitrogen deficiency caused leaf yellowing and translucency, it may also explain the adventitious root formation, which was observed only in hormone-free media. Nitrogen levels have been shown to influence adventitious root generation in *Pelargonium spp.* (Bellini et al., 2014). Endogenous plant hormones strongly influence adventitious root formation in dicotyledonous plants like tomato. Salicylic acid, auxins, cytokinins, jasmonic acid, and brassinosteroids enhance root generation. Among these, nicotine affects the synthesis of salicylic acid, auxins, and jasmonic acid (Cheng et al., 2021). This could also be why adventitious roots were observed. This cannot be confirmed, however, due to the lack of studies on the effect of nicotine in plant growth and development.

Conclusion

CN influences tomato seed germination in both hormone-supplemented and hormone-free media. Lower concentrations inhibit germination, while higher concentrations result in sprouting rates comparable to controls. Nicotine stress induces plant growth hormones that, combined with supplemented hormones, suppress overall seedling development. CN significantly alters seedling morphology in hormone-supplemented media, inhibiting both root and shoot growth, particularly lateral roots especially in higher concentrations. In hormone-free media, CN-treated seedlings exhibited nutrient deficiency and leaf translucency. Overall, CN has an inhibitory effect on plant growth and development. However, sprouting rates peaked at 100 μL/L in hormone-supplemented media and 50 μL/L in hormone-free media, suggesting CN leachate negatively impacts plant growth.

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