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Allelopathic potential of *Amaranthus spinosus* extract on the physiological activities of Talam 2 and Hypoma 2 peanut varieties

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ABSTRACT

Peanut (*Arachis hypogaea*) is an important legume crop and a vital source of plant based protein. However, its cultivation faces challenges from biotic stresses, including competition with allelopathic weeds like *Amaranthus spinosus*. Interestingly, allelochemicals from this weed can exhibit a hormetic effect, acting as biostimulants at specific concentrations. This study aimed to examine the allelopathic potential of various concentrations of *Amaranthus spinosus* extract on the physiological activity of two peanut varieties. The research was conducted from July to September 2025 at the Faculty of Agriculture Greenhouse, Universitas Sebelas Maret Surakarta, using a factorial randomized complete block design (RCBD). The first factor was peanut variety (Talam 2 and Hypoma 2), while the second factor was extract concentration (0%, 0.5%, 1%, 1.5%, and 2%). The results showed a significant interaction between variety and extract concentration on chlorophyll b and total chlorophyll content. The application of 0.5% extract concentration increased chlorophyll a content (0.6387 mg g^{-1}), stomatal aperture width (8.18 µm), and stomatal aperture length (17.36 µm). Meanwhile, 1% extract concentration increased stomatal conductance (0.2872 µmol m^{-2} s^{-1}) and transpiration rate (0.0290 µmol m^{-2} s^{-1}). Talam 2 variety showed the highest leaf greenness (47.29 SPAD). These findings demonstrate that the allelopathic effect of *A. spinosus* extract exhibits a biphasic hormetic response, where low concentrations function as biostimulants while higher concentrations induce physiological stress, with variety specific responses in peanut plants.

Keywords: allelochemical, biostimulant, hormesis, physiological, weed.

INTRODUCTION

Peanut (*Arachis hypogaea*) is a legume crop from the Fabaceae family that serves as the world's fourth most important oilseed crop and plays a crucial role in global food security (Guchi, 2015; Kombiok et al., 2012; L. Li et al., 2024). Additionally, peanuts represent one of the primary plant based protein sources (Duan et al., 2022).With high seed oil (46–58%) and protein (22–32%) content, peanuts have become a strategic crop in addressing malnutrition, enhancing vegetable oil self-sufficiency, and maintaining food security (Liu et al., 2024). In Indonesia, improving peanut productivity relies on developing superior varieties, such as Talam 2, known for its high adaptability to marginal lands also can yield up to 4 t ha^{-1} (Asis et al., 2022). Meanwhile, the Hypoma 2 variety also demonstrates advantages with a potential yield of 3.5 t ha^{-1}, resistance to leaf spot disease, high pod numbers, large seed size, and tolerance to drought conditions during the generative phase (Erliyana et al., 2015)**.** However, realizing the high yield potential of these varieties is constrained by biotic stresses, particularly weed competition. Weeds are a major problem throughout the peanut growth cycle (Budiastuti et al., 2024; El-Metwally and Saudy, 2021). Weeds exert allelopathic mechanisms and compete for growth resources. Peanuts, which grow relatively slowly in the early stages of planting, provide an ideal environment for weed growth (Grichar et al., 2021). With their low morphology and narrow canopy, peanut plants allow broadleaf weeds to become more competitive in acquiring light, especially during the early growth stages (Daramola et al., 2024; Mekdad et al., 2021). This competition disrupts physiological processes and can cause significant yield losses, reported to range from 15% to 84% (Mavarkar et al., 2015; Rahayu et al., 2025). These losses occur primarily because weeds reduce the availability of resources needed by peanut plants, particularly during critical growth phases. The critical period of competition between peanut plants and weeds occurs at 40–45 days after planting (DAP), varying with variety and environment (Shittu et al., 2022). Different peanut varieties possess varying levels of tolerance to weed stress. Consequently, developing varieties with tolerance to such biotic stress is a key breeding objective (Fan et al., 2020).

A significant weed in peanut cultivation is spiny amaranth (*Amaranthus spinosus* L.), known for its rapid growth and high competitive ability (Erida et al., 2021; Li et al., 2023). Beyond resource competition, *A. spinosus* suppresses crops allelopathically by releasing biochemicals that affect the growth and development of adjacent plants (Sarkar and Chakraborty, 2015; Sarker and Oba, 2019). Allelopathy refers to the physiological process where an organism, typically a plant, releases bioactive compounds into the environment that influence the germination, growth, or survival of neighboring plants. *A. spinosus* contains various bioactive compounds such as alkaloids, flavonoids, glycosides, phenolic acids, steroids, terpenoids, saponins, betalains, β-sitosterol, stigmasterol, rutin, catechu tannin, and other compounds (Carvalho et al., 2019; Ekeke et al., 2019; Prajitha and Thoppil, 2016). In general, the chemical compounds found in the leaves of *A. spinosus* belong to the polyphenol group, including gallic acid, caffeic acid, vanillic acid, rutin, and coumaric acid (Rjeibi et al., 2017). The interaction of allelochemicals with plants exhibits a biphasic phenomenon known as hormesis. Hormesis describes the dose-response relationship where low concentrations of a stressor agent have a stimulatory effect, while high concentrations are inhibitory (Perveen et al., 2021a). This dose dependent response means that allelochemicals can stimulate plant growth at low concentrations but inhibit it at higher ones (Choudhary et al., 2023), presenting a dual potential for their use in agriculture. The stimulatory effect of allelochemicals can be utilized as bio-fertilizers hayati (Behera et al., 2021), while the inhibitory effect can be used as a natural herbicide to suppress the growth of other weeds.

Previous studies have consistently demonstrated the hormetic effects of various plant extracts. For example, a low concentration (1%) of *Annona muricata* extract was shown to stimulate the growth and biochemical parameters of *Vigna radiata* seedlings, such as seedling length, pigments, starch, protein, amino acids, carbohydrates, peroxidase, and catalase activity, while higher concentrations were inhibitory (Kannan and Palayian, 2022). A similar pattern was reported by Ashokkuma et al. (2024) for *Parthenium hyterophorus* extract, where a 5% concentration significantly inhibited seed germination, impaired root growth, and reduced root length, shoot length, and biomass in mung bean and finger millet compared to 0, 2, and 3% concentrations. Furthermore, allelopathic extracts of *Lantana camara* at concentrations of 1, 3, and 5% reduced the germination rate, chlorophyll content, and protein content in peanuts (Gaikwad et al., 2023). These findings confirm the universality of the hormesis phenomenon. However, most of these studies have focused on inhibitory responses or have only involved a single test plant species. An in depth exploration of the stimulatory physiological responses at low concentrations, particularly in commercial crops like peanuts across different varieties, remains very limited. Therefore, this study aims to investigate the hormetic response of various concentrations of *A. spinosus* extract on the physiological activity of two peanut varieties with different genetic backgrounds, namely the Talam 2 and Hypoma 2 varieties. It is hypothesized that a low concentration (1%) of *A. spinosus* extract will elicit the greatest stimulatory effect on the physiological performance of both peanut varieties, with the Talam 2 variety anticipated to demonstrate a more pronounced response compared to Hypoma 2 due to its genetic background. The findings from this study are expected to provide a scientific basis for the utilization of *A. spinosus* extract as a biostimulant at low doses or as a bioherbicide at high doses, with selectivity based on the crop variety.

MATERIAL AND METHODS

Research location and environmental conditions

The study was conducted from July to September 2025. All plant cultivation and treatment applications were carried out in a greenhouse at the Faculty of Agriculture, Universitas Sebelas Maret, located at 7°33’41.7” S, 110°51’32.6” E, with an elevation of 96 meters above sea level. Throughout the experimental period, the average air temperature inside the greenhouse was maintained between 25–32 °C, with relative humidity ranging from 65% to 80%. Light intensity was monitored daily using a lux meter. The preparation of plant extracts and all physiological analyses were performed at the Plant Physiology and Biotechnology Laboratory, Universitas Sebelas Maret.

Plant material and growth conditions

Seeds of two peanut varieties (*A. hypogaea*), Talam 2 and Hypoma 2, were obtained from the Legume Crops Instrument Standard Testing Center (BPSI Tanaman Aneka Kacang) in Malang, Indonesia. The plants were grown in 40 × 40 cm polybags filled with latosol soil, which was characterized prior to the experiment (organic C: 1.15%, total N: 0.28%, available P: 31.04%, exchangeable K: 0.22 meq%, pH: 6.61). A standard dose of organic fertilizer was mixed into the soil before planting.

Experimental design

The research was structured using a factorial Randomized Complete Block Design (RCBD) (Figure 1). The first factor consisted of peanut variety, namely Talam 2 (V1) and Hypoma 2 (V2). The second factor was the concentration of *A. spinosus* extract, with five levels: 0% (control, K0), 0.5% (K1), 1% (K2), 1.5% (K3), and 2% (K4). Each of the 10 treatment combinations was replicated three times, resulting in 30 experimental units. Each unit consisted of 5 polybags, giving a total of 150 polybags. The blocks were arranged to account for any potential environmental gradient within the greenhouse.

Preparation of *A. spinosus* extract

The extraction procedure was adapted from established protocols with modifications (Gaikwad et al., 2023; Setiawan et al., 2024). The collected *A. spinosus* plants were thoroughly washed under running tap water to remove soil and debris. The plants were then separated into roots, stems, leaves, and flowers. The plant parts were first sun dried for three days to reduce moisture, followed by oven drying at 40 °C for 72 hours in brown paper envelopes to prevent the degradation of heat sensitive compounds (Chuo et al., 2022). The dried plant matter was pulverized in a blender into a powder and sieved to obtain a fine powder (Yau et al., 2022). A total of 100 g of the plant powder was macerated in 1000 mL of 70% aqueous methanol (1:10 w/v) in a dark glass container for 72 hours (Irfan et al., 2022). The macerate was filtered, and then concentrated using a rotary evaporator at 40 °C under reduced pressure to remove the methanol solvent. The resulting aqueous crude extract was then diluted with distilled water to prepare the treatment solutions with concentrations of 0% (control), 0.5%, 1%, 1.5%, and 2% (w/v). Each pot received a 100 mL soil drench of its respective treatment, applied evenly around the root zone using a graduated measuring cup. To target the critical growth period for weed competition in peanuts, applications were made at 2, 4, and 6 weeks after planting (WAP) (Osunleti, 2022). The process is summarized in Figure 2.

Data collection on physiological parameters

All physiological parameters were measured at 7 WAP, during the peak vegetative growth stage. For all leaf-based measurements, the third fully expanded leaf from the top of the main stem was sampled, unless otherwise specified. The specific procedures for each parameter are detailed below (Figure 3).

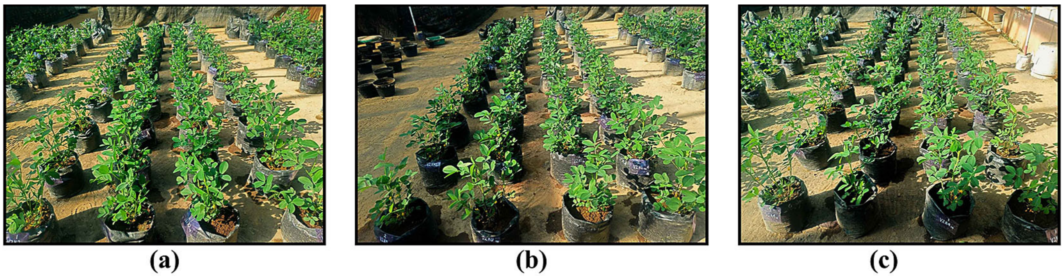
(a) (b) (c)

Figure 1. Experimental design (a) block 1 (b) block 2 (c) block 3

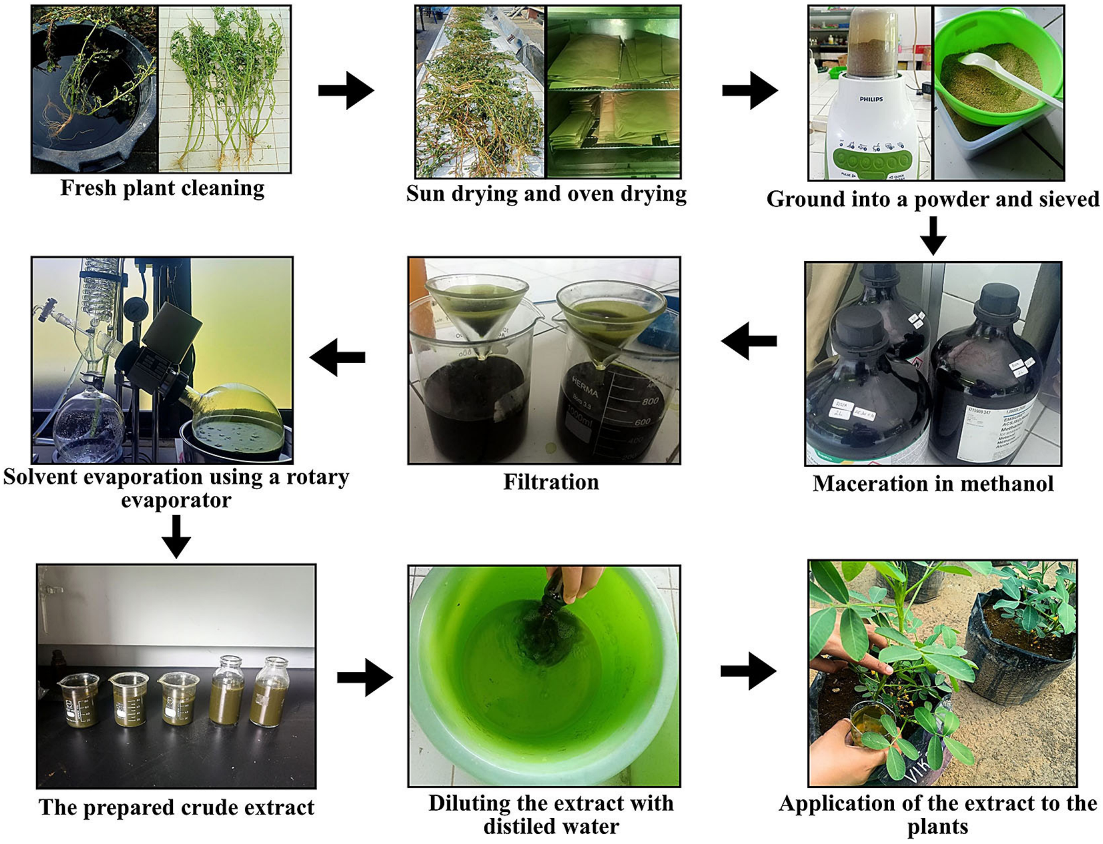

Figure 2. Schematic flowchart of the extraction process of *A. spinosus*

Chlorophyll content

Chlorophyll was quantified spectrophotometrically using the Arnon method (Doddavarapu et al., 2021). Briefly, 1 g of fresh leaf sample was homogenized in 20 mL of 80% acetone. The absorbance of the supernatant was measured at 663 nm and 645 nm. The chlorophyll content was calculated using the Arnon method as follows:

$$\text{Chlorophyll } A = (12.7 \times A\,663 - 2.69 \times A\,645) \times v/w \times 1000 \quad (1)$$

$$\text{Chlorophyll } B = (22.9 \times A\,645 - 4.68 \times A\,663) \times v/w \times 1000 \quad (2)$$

$$\text{Total chlorophyll} = (20.2 \times A\,645) + (8.02 \times A\,663) \times v/w \times 1000 \quad (3)$$

where: v – supernatant volume, w – leaf weight.

Leaf greenness (SPAD value)

Leaf greenness was measured using a SPAD-502 meter. Measurements were taken at three different points on the leaf blade, avoiding the midrib, and the average value was recorded.

Stomatal characteristics (density and aperture)

Stomatal impressions were taken from the abaxial (lower) leaf surface between 09:00 and 11:00 a.m. using clear nail polish (Millstead et al., 2020). The dried polish was peeled off with transparent tape, mounted on a glass slide, and visualized under a light microscope at 10 and 40x magnification. Stomatal density was determined by counting the number of stomata in five random fields of view per leaf using the Image Raster application. The result was expressed as the number of stomata per mm^2. Stomatal Aperture (width and length) was measured from the micrographs using Image Raster.

Stomatal conductance and transpiration rate

Stomatal conductance (gs) and transpiration rate (E) were measured using a portable photosynthesis system. Measurements were taken between 09:00 and 11:00 a.m. under clear sky conditions on the central leaflet of the second fully developed leaf, avoiding the leaf margins and major veins (Cseresnyés et al., 2024; Thangthong et al., 2021). The instrument's chamber environment was set to match ambient CO_2 and light conditions.

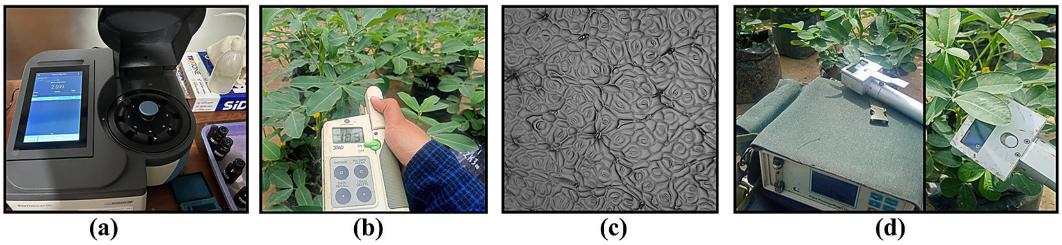

Figure 3. Data collection process for (a) chlorophyll content, (b) leaf greenness, (c) stomatal characteristics, (d) stomatal conductance and transpiration rate

Statistical analysis

All statistical analyses were performed using SPSS. The dataset analyzed included the raw data from all three replicates (n = 3) for each treatment combination. This was based on a factorial design of 2 peanut varieties (Talam 2 and Hypoma 2) × 5 concentrations of *A. spinosus* extract (0, 0.5, 1, 1.5, and 2%), resulting in a total of 30 individual data points for the analysis of each physiological parameter. The variables were analyzed using a two-way Analysis of Variance (ANOVA) to determine the main effects of peanut variety, extract concentration, and their interaction on the physiological parameters, which included chlorophyll a, b, and total content, leaf greenness, stomatal density, stomatal aperture width, stomatal aperture length, stomatal conductance, and transpiration rate. When statistically significant main or interaction effects were observed ($p<0.05$), Duncan's Multiple Range Test (DMRT) was applied to identify specific differences between treatment means. Significance was established at $p<0.05$. The results of these analyses are presented in the following sections, with mean values accompanied by their standard deviation (SD) and separated by different lowercase letters to denote significant differences. To model the response of the physiological variables to the extract concentration, polynomial regression analysis was conducted to determine the optimal concentration for each variety.

RESULT AND DISCUSION

Chlorophyll a

Chlorophyll a is the main pigment that drives photosynthesis, located in the reaction centers of both Photosystem I and II, where it plays a direct role in the crucial primary photochemical event. The complete dataset for chlorophyll a content across all treatment combinations is presented in Table 1. The mean chlorophyll a content across all treatment was 0.6250 mg g^{-1}, with values ranging from 0.6123 to 0.6387 mg g^{-1}. These data were subjected to a two-way ANOVA to examine the effects of variety, *A. spinosus* extract concentration, and their interaction. The result showed

Table 1. Effect of several concentrations of *A. spinosus* extract on the chlorophyll a content (mg g^{-1}) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	0.6124	0.6091	0.6008	0.6075
Talam 2 + 0.5%	0.6245	0.6428	0.6187	0.6287
Talam 2 + 1%	0.6137	0.6220	0.5977	0.6111
Talam 2 + 1.5%	0.6247	0.6182	0.6373	0.6268
Talam 2 + 2%	0.6167	0.6232	0.6517	0.6305
Hypoma 2 + 0%	0.6190	0.6203	0.6120	0.6171
Hypoma 2 + 0.5%	0.6260	0.6808	0.6394	0.6487
Hypoma 2 + 1%	0.6200	0.6184	0.6386	0.6257
Hypoma 2 + 1.5%	0.6318	0.6109	0.6040	0.6156
Hypoma 2 + 2%	0.6466	0.6197	0.6475	0.6379
Average	0.6235	0.6265	0.6248	0.6250

that the concentration of the *A. spinosus* extract had a significant effect on chlorophyll a content ($p<0.05$). In contrast, the effect of the peanut variety was not statistically significant ($p>0.05$). Furthermore, the interaction between variety and extract concentration was also not significant ($p>0.05$) (Table 2). Given the significant main effect of concentration, a Duncan's post-hoc test was conducted to compare means across different concentration levels. The post-hoc test indicated that the application of the *A. spinosus* extract at a concentration of 0.5% resulted in the highest chlorophyll a content (0.6387±0.0226 mg g^{-1}), which was significantly higher than the control (0.6123±0.0071 mg g^{-1}) and other concentrations (Figure 4).

The content of chlorophyll a, as the primary pigment in the photosynthetic reaction (Lokstein et al., 2021), exhibited a hormetic response pattern to the application of the *A. spinosus* extract. This pattern is characterized by stimulation at low concentrations and inhibition at high concentrations (Perveen et al., 2021b). This increase suggests that at low concentrations, the allelochemical compounds in the extract act as elicitors that stimulate chlorophyll synthesis. *A. spinosus* leaves are known to contain several active compounds, including phenolic acids such as protocatechuic acid, ferulic acid, p-coumaric acid, chlorogenic acid, and gallic acid, as well as flavonoids like kaempferol, rutin, myricetin, naringenin, apigenin, quercetin, and catechin (Kar and Bhattacharjee, 2022). At low concentrations, these flavonoid compounds can donate electrons, their antioxidant protection mechanism is achieved by donating electrons from functional hydroxyl groups to stabilize free radicals (Šamec et al., 2021). This activity results in a temporary increase in reactive oxygen species (ROS) to a non damaging level. These low level ROS serve as signaling agents that elicit defense mechanisms and adaptive responses to stress conditions (Kesawat et al., 2023), including the regulation of growth and gene transcription to cope with multifactorial pressure (Thiruvengadam et al., 2024). This mechanism allows the plant to adapt to the mild stress induced by the extract, thereby enabling it to maintain and even enhance chlorophyll synthesis. This finding aligns with the research by Gomaa et al., (2023) which reported that phenolic compounds at low concentrations can produce positive effects on growth and cell metabolism, while inhibition occurs at higher concentrations.

Regarding the effect of variety, the chlorophyll a values in the Talam 2 variety (0.6209±0.0146 mg g^{-1}) and the Hypoma 2 variety (0.6290±0.0194 mg g^{-1}) did not show a significant difference (Figure 4 and Table 2). This is most likely due to insufficient genetic diversity between the two varieties concerning chlorophyll a synthesis and regulation. Although they are different

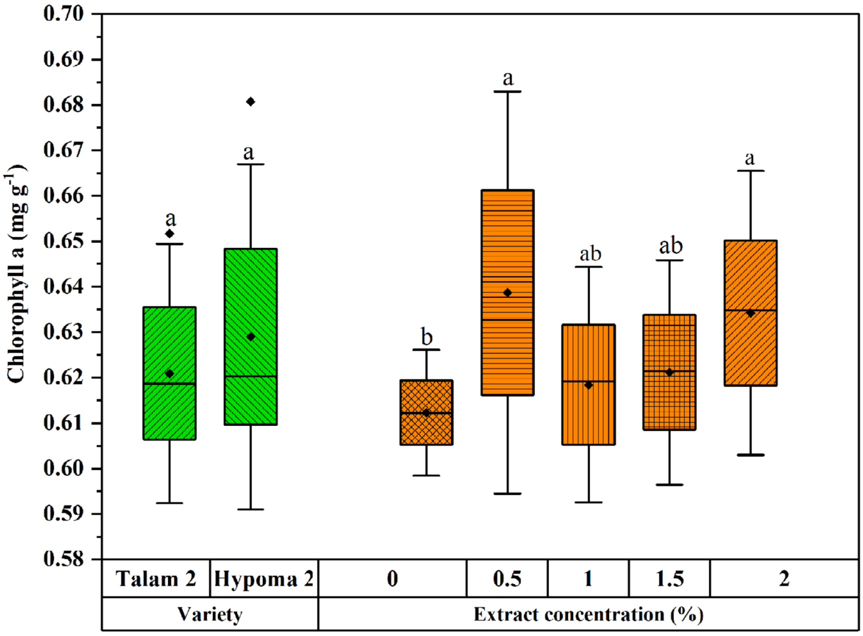

Figure 4. Chlorophyll a content of peanut. Values with the same letter are not significantly different based on the Duncan's Multiple Range Test ($\alpha = 0.05$)

varieties, the genes encoding key enzymes in chlorophyll a biosynthesis, such as chlorophyll synthase and protochlorophyllide reductase, are likely homologous or exhibit highly similar expression. Both varieties belong to the Spanish type and are thus genetically closely related (Rahmianna et al., 2020). Consequently, the baseline photosynthetic capacity of the two varieties is equivalent. Furthermore, the uniform environmental conditions throughout the study, such as the availability of nitrogen and magnesium, light intensity, and an optimal water supply further minimized variation. Under these non stress conditions, both genotypes were able to fully express their genetic potential, reaching a maximal and statistically similar level of chlorophyll a.

Chlorophyll b

Chlorophyll b is an accessory pigment in the photosynthetic antenna complexes, primarily responsible for absorbing light energy in the blue and red orange spectra and transferring it efficiently to chlorophyll a to maximize the light capture for photosynthesis. The complete dataset for chlorophyll b content across all treatment combinations is presented in Table 3. The mean of chlorophyll b content across all treatment was 1.0665 mg g^{-1}, with values ranging from 1.0251 to 1.0932 mg g^{-1}. These data were first subjected to a two-way ANOVA to examine the effects of variety, *A. spinosus* extract concentration, and their interaction. The analysis revealed a significant interaction ($p<0.05$) between peanut varieties and the concentration of *A. spinosus* extract on chlorophyll b content (Table 2). To further characterize the nature of this interaction, polynomial regression analysis was performed separately for each variety. This analysis showed distinctly different response patterns between the two varieties (Figure 5). In the Talam 2 variety, a negative quadratic pattern indicated that increasing the *A. spinosus* extract initially raised the chlorophyll b content, but beyond a certain concentration, further increases led to a decline. The Talam 2 variety exhibited a hormetic response, with an optimum concentration at 0.915%. At this concentration, the chlorophyll b content reached its maximum value of 1.1162 mg g^{-1}. Conversely, in the Hypoma 2 variety, the application of the *A. spinosus* extract resulted in a positive quadratic pattern, characterized by an initial decrease followed by a tendency to increase at higher concentrations. The Hypoma 2 variety had a critical point at 1.368%, however, this point represents a minimum, indicating that within the tested concentration range (0–2%), this variety did not reach a maximum optimum point. This differential response is strongly suspected to

Table 2. Mean values of chlorophyll a, chlorophyll b, total chlorophyll, and leaf greenness in peanut leaves under different treatments

Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Leaf greenness (SPAD value)
Variety				
Talam 2	0.6209±0.0146a	1.0784±0.0975a	1.6993±0.0967a	47.29±1.96a
Hypoma 2	0.6290±0.0194a	1.0546±0.0857a	1.6836±0.0872a	45.35±1.27b
A.spinosus extract concentration (%)				
0	0.6123±0.0071a	1.0932±0.0975a	1.7005±0.0468a	46.26±1.46a
0.5	0.6387±0.0226a	1.0861±0.0857a	1.7248±0.0701a	47.36±2.09a
1	0.6184±0.0132ab	1.0479±0.1584a	1.6663±0.1472a	46.19±2.14a
1.5	0.6212±0.0126ab	1.0803±0.0864a	1.7014±0.0870a	45.56±2.54a
2	0.6342±0.0159a	1.0251±0.0768a	1.6593±0.0880a	46.23±1.15a
Interaction	-	+	+	-
p-value of variety	0.173	0.371	0.569	0.06
p-value of *A. spinosus* extract concentration	0.044	0.436	0.520	0.498
p-value of interaction	0.504	0.010	0.019	0.750
CV (%)	1.97	6.88	5.71	24.89

Note: values followed by the same letter are not significantly different according to Duncan's multiple range test ($\alpha = 0.05$). A plus sign (+) indicates a positive interaction, while a minus sign (-) indicates no interaction.
CV (%) – coefficient of variance.

Table 3. Effect of several concentrations of *A. spinosus* extract on the chlorophyll b content (mg g^{-1}) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	1.0503	1.0909	1.0353	1.0588
Talam 2 + 0.5%	1.0011	1.0689	1.1606	1.0769
Talam 2 + 1%	1.0520	1.1405	1.3020	1.1648
Talam 2 + 1.5%	0.9086	1.1064	1.1406	1.0519
Talam 2 + 2%	1.0408	0.9276	1.1506	1.0397
Hypoma 2 + 0%	1.1105	1.1219	1.1503	1.1276
Hypoma 2 + 0.5%	1.0383	1.1246	1.1232	1.0953
Hypoma 2 + 1%	0.9257	1.0082	0.8588	0.9309
Hypoma 2 + 1.5%	1.1049	1.1337	1.0874	1.1087
Hypoma 2 + 2%	1.0123	0.9690	1.0503	1.0105
Average	1.0245	1.0692	1.1059	1.0665

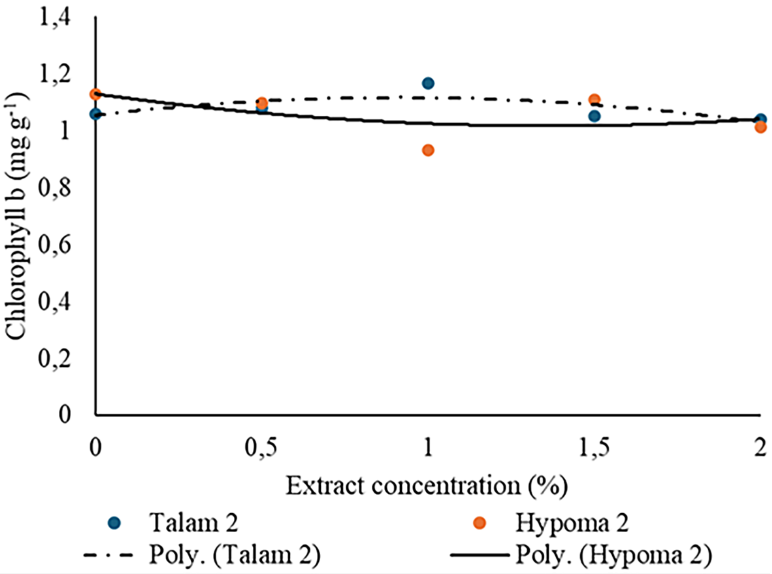

Figure 5. The interaction between peanut variety and extract concentration on the chlorophyll b content of peanut. Talam 2 $y = -0.0747x^2+0.1367x+1.0537$ $R^2 = 0.525$; Hypoma 2 $y = 0.0601x^2-0.1644x+1.1288$ $R^2 = 0.296$

occur because chlorophyll b, which functions to absorb light at around 450 nm a spectral region not efficiently absorbed by chlorophyll a has an expression dependent on the specific genotype of each variety (Khan et al., 2023). This finding aligns with the report by (Zou et al., 2022) on the genetic variation of chlorophyll content in legume species. The response patterns are attributed to the presence of several phenolic compounds in the *A. spinosus* extract. In the Talam 2 variety, at low concentrations, phenolic compounds can act as antioxidants, helping plants counteract the reactive oxygen species (ROS) generated from normal metabolic processes (Kumar et al., 2023). At higher concentrations, the chlorophyll b content tended to decrease. This decline is caused by compounds in *A. spinosus*, such as saponins and alkaloids, which at high concentrations can disrupt cell membrane integrity, inhibit key enzymes, and induce severe oxidative damage (Jabeen et al., 2023).

In the Hypoma 2 variety, at higher concentrations, activated detoxification mechanisms and antioxidant systems likely enabled this variety to utilize antioxidant compounds from the extract to neutralize oxidative stress, consequently leading to a recovery in chlorophyll b content. The protective effect of plant extracts against abiotic stress stems from their complex mix of secondary metabolites, which activate multiple metabolic pathways. This growth regulating benefit is not due to a single compound, but arises from the synergistic interaction of various constituents like plant growth regulators, antioxidants, and osmoprotectants (Han et al., 2024). The differential response of the Talam 2 and Hypoma 2 varieties to the *A. spinosus* extract can be attributed to one of two factors, either genetic variations in the expression of enzymes specific to the chlorophyll biosynthesis pathway, or differences in the signal transduction systems that detect the elicitors in the extract

Chlorophyll total

Total chlorophyll represents the combined concentration of chlorophyll a and b in plant tissues, serving as a direct indicator of the overall density of the photosynthetic apparatus and the plant's capacity to harvest light energy. The complete dataset for total chlorophyll content across all treatment combinations is presented in Table 4. The mean of total chlorophyll content across all treatment was 1.69 mg g^{-1}, with values ranging from 1.66 to 1.72 mg g^{-1}. These data were first subjected to a two-way ANOVA to examine the effects of variety, *A. spinosus* extract concentration, and their interaction. The results regarding total chlorophyll content reinforce the previous findings for chlorophyll b, demonstrating a significant interaction ($p<0.05$) between the variety and the concentration of the *A. spinosus* extract (Table 2). To further characterize the nature of this interaction, polynomial regression analysis was performed separately for each variety. This analysis revealed distinctly different response patterns between the two varieties (Figure 6). The Talam 2 variety exhibited a negative quadratic pattern, indicating the presence of a maximum point. In contrast, the Hypoma 2 variety displayed a positive quadratic pattern, signifying a minimum point. The response patterns observed in total chlorophyll content indicate varietal specific hormetic effects. In the Talam 2 variety, increasing the concentration of *A. spinosus* extract initially enhanced total chlorophyll content. However, beyond a certain concentration, further increases in the extract led to a decline in total chlorophyll. This result demonstrates a hormetic pattern characterized by stimulation at low concentrations and inhibition at high concentrations, peaking at a concentration of 0.974%. Conversely, the Hypoma 2 variety exhibited a different pattern. Within the tested concentration range, the plants did not show a clear stimulatory response but rather a general trend of decreasing total chlorophyll content. The highest total chlorophyll content for Hypoma 2 was actually observed at the 0% concentration (1.70 mg g^{-1}), indicating that this variety is less responsive to the spiny amaranth extract treatment.

This interaction confirms that the physiological response of peanut plants to the spiny amaranth extract treatment is highly dependent on the specific genotype of each variety. Leaf chlorophyll content serves as a key indicator of a plant's photosynthetic capacity and productivity (Elsayed et al., 2023), and total chlorophyll content is not universal but is significantly influenced by varietal differences (El-Hendawy et al., 2021).

Table 4. Effect of several concentrations of *A. spinosus* extract on the total chlorophyll content (mg g^{-1}) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	1.6627	1.7001	1.6361	1.6663
Talam 2 + 0.5%	1.6257	1.7117	1.7793	1.7055
Talam 2 + 1%	1.6657	1.7625	1.8997	1.7760
Talam 2 + 1.5%	1.5333	1.7247	1.7779	1.6786
Talam 2 + 2%	1.6575	1.5508	1.8023	1.6702
Hypoma 2 + 0%	1.7295	1.7422	1.7623	1.7447
Hypoma 2 + 0.5%	1.6643	1.8053	1.7625	1.7440
Hypoma 2 + 1%	1.5457	1.6266	1.4974	1.5566
Hypoma 2 + 1.5%	1.7367	1.7446	1.6914	1.7242
Hypoma 2 + 2%	1.6588	1.5887	1.6978	1.6484
Average	1.6480	1.6957	1.7307	1.6915

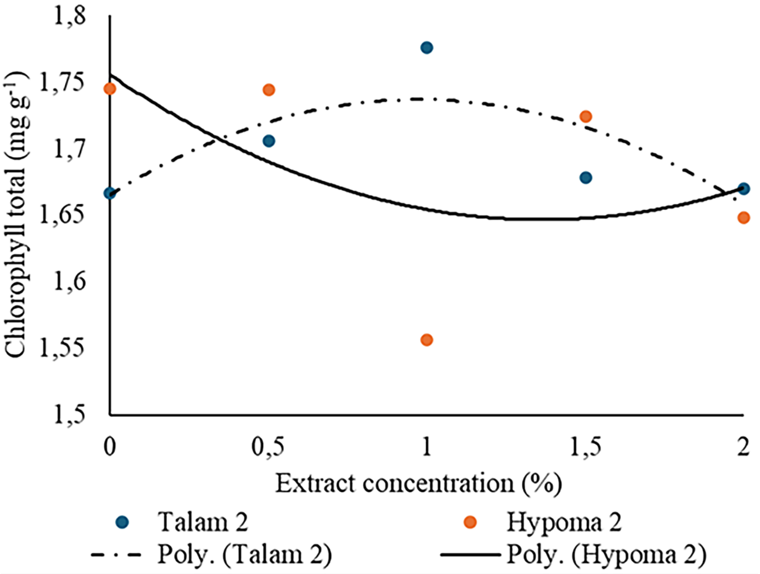

Figure 6. The interaction between peanut variety and extract concentration on the total chlorophyll content peanut. Talam 2 $y = -0.0752x^2+0.1465x+1.6656$ $R^2 = 0.6015$; Hypoma 2 $y = 0.0585x^2-0.1595x+1.7553$ $R^2 = 0.2842$

The differing total chlorophyll responses between the two varieties demonstrate that the reaction to the spiny amaranth extract is highly specific and dependent on the genotype of each variety. The divergent responses observed in chlorophyll concentration are caused by stimulatory or inhibitory effects, reduced mineral uptake, and abnormalities in the chlorophyll regulatory system (Siyar et al., 2019). Specific allelochemicals can contribute to either an increase or decrease in chlorophyll synthesis. *A. spinosus* leaves contain a variety of allelochemicals, including alkaloids (50.82 g $100g^{-1}$), flavonoids (50.88 g 100 g^{-1}), phenolics (19.27 g 100 g^{-1}), glycosides (10.07 g 100 g^{-1}), oxalate (2.84 ppm), tannins (7.961 ppm), saponins (5.118 ppm), and trypsin inhibitor (1.98 ppm) (Ekeke et al., 2019). The decrease in total chlorophyll content is likely caused by chlorophyll degradation or reduced synthesis due to flavonoids, terpenoids, or other phytochemicals present in the weed extract (Mushtaq et al., 2020). The reduction in chlorophyll content may also result from the breakdown of chlorophyll molecules by allelochemicals targeting the pyrrole ring and its phytyl chain (Mushtaq et al., 2020; Pareek et al., 2017).

Leaf greenness

Leaf greenness, as a non destructive proxy for chlorophyll content, was measured to provide a rapid, integrative assessment of the treatment effects on the plant's photosynthetic capacity throughout the growth period, complementing the destructive measurements of specific chlorophyll a and b fractions. The complete dataset for leaf greenness across all treatment combinations is presented in Table 5. The mean of leaf greenness across all treatment was 46.32 SPAD, with values ranging from 45.35 to 47.35 SPAD (Figure 7). These data were subjected to a two-way ANOVA to examine the effects of variety, *A. spinosus* extract concentration, and their interaction. The results showed that the main effect of peanut variety on leaf greenness was statistically significant ($p<0.05$). In contrast, the main effect of *A. spinosus* extract concentration ($p>0.05$) and the interaction between variety and concentration ($p>0.05$) were not statistically significant (Table 2). Following the finding that variety had a significant effect, a comparison of the means reveals that the Talam 2 variety exhibited a higher leaf greenness value (45.35±1.27 SPAD). This genetic variation in leaf greenness suggests differences in chlorophyll density or leaf structure between the two varieties, which can affect photosynthetic capacity and overall productivity (Cao et al., 2022). Regarding the effect of extract concentration, the non-significant ANOVA result is reflected in the SPAD values, which remained relatively stable across all treatments, ranging

Table 5. Effect of several concentrations of *A. spinosus* extract on the leaf greenness (SPAD values) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	45.40	45.30	49.15	46.62
Talam 2 + 0.5%	49.90	48.75	47.55	48.73
Talam 2 + 1%	45.15	46.15	49.95	47.08
Talam 2 + 1.5%	45.00	45.80	50.45	47.08
Talam 2 + 2%	45.95	48.25	46.60	46.93
Hypoma 2 + 0%	46.00	45.55	46.16	45.90
Hypoma 2 + 0.5%	43.95	47.90	46.10	45.98
Hypoma 2 + 1%	46.65	43.50	45.75	45.30
Hypoma 2 + 1.5%	43.50	44.85	43.75	44.03
Hypoma 2 + 2%	45.95	45.85	44.80	45.53
Average	47.75	46.19	47.03	46.32

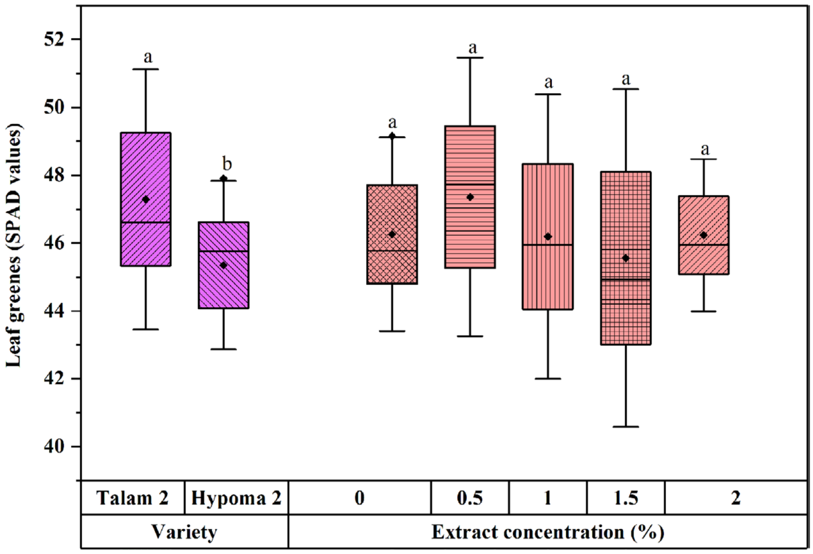

Figure 7. Leaf greenness of peanut. Values with the same letter are not significantly different based on the Duncan's Multiple Range Test (α = 0.05)

from 45.46 to 47.36. Unlike other physiological parameters that exhibited a hormetic pattern, the application of *A. spinosus* extract at various concentrations (0–2%) did not exert a significant influence on SPAD values. This stability in SPAD values indicates that, in the short term, the genetic and anatomical characteristics of the leaves (such as leaf thickness and mesophyll structure) have a more dominant influence than the allelopathic stress from the extract.

Leaf greenness measured with a SPAD meter reflects the total chlorophyll content in the leaf tissue. SPAD values are influenced not only by chlorophyll content but also by leaf thickness, mesophyll structure, and other anatomical characteristics that may remain unaffected by the extract treatment in the short term (Cao et al., 2022). The contrasting results between the stable SPAD values and the significantly affected measured chlorophyll content (a, b, and total) reveal the complexity of the plant's physiological response. The stability of the SPAD values indicates that the integrity of the leaf's anatomical structure such as mesophyll thickness and cell arrangement, which also influence SPAD readings remained relatively undisturbed by the short-term allelopathic treatment. Within the observed concentration range and timeframe, the allelochemicals were more effective at disrupting the biochemical function of chlorophyll than damaging the physical structure of the leaves (Rahaman et al., 2022).

Stomatal density

Stomatal density is defined as the quantity of stomata present per unit area on the leaf epidermis, directly influencing the rates of photosynthesis and transpiration. The mean values for stomatal density across all treatment combinations is presented in Table 6. The complete dataset of stomatal density across all treatment was 168.36 unit mm^{-2}, with values ranging from 164.16 to 173.95 unit mm^{-2}. These data were subjected to a two-way ANOVA to examine the effects of variety, *A. spinosus* extract concentration, and their interaction. The results showed that the main effect of variety was not statistically significant ($p>0.05$). Similarly, the main effect of *A. spinosus* extract concentration ($p>0.05$) and the interaction between variety and concentration ($p>0.05$) were also not statistically significant (Table 7). The stomatal density across all concentration treatments was relatively stable, ranging from 164.16±14.59 to 173.95±19.98 unit mm^{-2}. Likewise, no significant difference was observed between the Talam 2 (170.32± 17.91 unit mm^{-2}) and Hypoma 2 (166.40±15.64 unit mm^{-2}) varieties. This indicates that neither varietal differences nor the application of A. spinosus extract at various concentrations (0–2%) had a significant effect on the stomatal density of peanut leaves.

Stomata, which are pores in the leaf epidermis consisting of an opening, a pair of guard cells, and subsidiary cells, play a crucial role in regulating gas exchange and plant transpiration (Windarsih et al., 2022). Stomatal density is an anatomical trait largely determined by the plant genotype and is established during the early developmental stages of the leaf. This characteristic is relatively stable and not easily altered as a short-term response to biotic or chemical stresses, such as the application of allelopathic extracts. The development of stomata progresses through a multi stage sequence, commencing with the initial cell divisions that establish meristemoid mother cells and culminating in the formation of specialized guard cell structures (Falquetto-Gomes et al., 2024). The differentiation and formation of stomata in leaf primordia are significantly influenced by environmental factors specifically during the stomatal development phase (Pérez-Bueno et al., 2022). The application of *A. spinosus* extract likely influenced short term physiological responses, such as the regulation of stomatal aperture, a reversible process mediated by modifications in guard cell turgor pressure, but did not affect anatomical characteristics like stomatal density, which is predetermined by genetic factors (Hasanuzzaman et al., 2023).

Stomatal aperture width

Stomatal aperture width is a measurement that determines the size of the pore between two guard cells, directly influencing the rate of gas exchange (CO_2, O_2, and H_2O) between the leaf and the atmosphere (Mishra et al., 2025). The complete dataset for stomatal aperture width across all treatment combinations is presented in Table 8. The mean of stomatal aperture width across all treatment was 7.26 µm, with values ranging from 6.77 to 8.18 µm. These data were subjected to a two-way ANOVA to examine the effects of variety, *A. spinosus* extract concentration, and their interaction. The results revealed a

Table 6. Effect of several concentrations of *A. spinosus* extract on the stomatal density (unit mm^{-2}) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	168.85	176.19	157.84	167.63
Talam 2 + 0.5%	168.85	171.30	161.51	167.22
Talam 2 + 1%	156.62	151.72	174.97	161.10
Talam 2 + 1.5%	171.30	201.89	139.49	170.89
Talam 2 + 2%	171.30	211.68	171.30	184.76
Hypoma 2 + 0%	139.49	179.86	162.73	160.69
Hypoma 2 + 0.5%	189.65	140.71	171.30	167.22
Hypoma 2 + 1%	145.60	176.19	179.86	167.22
Hypoma 2 + 1.5%	165.18	183.53	172.52	173.74
Hypoma 2 + 2%	151.72	166.40	171.30	163.14
Average	162.86	175.95	166.28	168.36

Table 7. Mean values of stomatal density, stomatal aperture width, stomatal aperture length, stomatal conductance, and transpiration rate of peanut under different treatments

Treatment	Stomatal density (unit mm^{-2})	Stomatal aperture width (μm)	Stomatal aperture length (μm)	Stomatal conductance (μmol m^{-2} s^{-1})	Transpiration rate (μmol m^{-2} s^{-1})
Variety					
Talam 2	170.32±17.91a	7.31±0.77a	15.43±1.14a	0.2380±0.1209a	0.0223±0.0131a
Hypoma 2	166.4±15.64a	7.21±0.61a	15.13±1.57a	0.1972±0.0936a	0.0189±0.0106a
A.spinosus extract concentration (%)					
0	164.16±14.59a	7.27±0.79b	15.17±0.49b	0.1171±0.0798b	0.0106±0.0074b
0.5	167.72±15.97a	8.18±0.43a	17.36±1.15a	0.1940±0.0591ab	0.0169±0.0047ab
1	164.16±14.59a	7.19±0.38b	14.68±0.47b	0.2872±0.0904a	0.0290±0.0122a
1.5	172.32±2.64a	6.77±0.35b	15.06±0.94b	0.2323±0.1350a	0.0216±0.0138ab
2	173.95±19.98a	6.89±0.41b	14.14±0.88b	0.2575±0.1045a	0.0250±0.0123a
Interaction	-	-	-	-	-
p-value of variety	0.565	0.594	0.317	0.202	0.322
p-value of *A. spinosus* extract concentration	0.824	0.001	0.0001	0.025	0.026
p-value of interaction	0.707	0.307	0.219	0.804	0.721
CV (%)	19.23	18.60	20.77	18.12	6.48

Note: values followed by the same letter are not significantly different according to Duncan's multiple range test ($\alpha = 0.05$). A plus sign (+) indicates a positive interaction, while a minus sign (-) indicates no interaction.
CV (%) – coefficient of variance.

statistically significant main effect of extract concentration on stomatal aperture width ($p<0.05$). In contrast, the main effect of variety ($p>0.05$) and the interaction between variety and concentration ($p>0.05$) were not statistically significant (Table 7). Given the significant effect of concentration, a Duncan's post-hoc test was conducted to compare means across different concentration levels. The post-hoc test indicated that treatment with a 0.5% concentration resulted in the highest stomatal aperture width (8.18±0.43 μm), which was significantly larger than all other treatments. Conversely, increasing the concentration of the extract to 1%, 1.5%, and 2% led to a significant reduction in stomatal aperture width, with values of 7.19±0.38 μm, 6.77±0.35 μm, and 6.89±0.41 μm, respectively, all lower than the control.

This finding indicates a hormetic effect, where exposure to a low dose of the extract acts as a beneficial, mild stressor, while higher doses are inhibitory (Cheng et al., 2024). The increase at the 0.5% concentration is hypothesized to occur because bioactive compounds in the extract, such as polyphenols, flavonoids, and alkaloids (Al-Tamimi et al., 2021), act as signaling molecules that stimulate stomatal opening at low concentrations. This mechanism is supported by research from (Martinez-Alonso et al., 2022) which reported that flavonoids can suppress abscisic acid (ABA) stress signaling, stimulate stomatal opening, and reduce ROS concentrations, thereby protecting membranes from damage. A wider stomatal opening has the potential to increase transpiration rates and CO_2 assimilation, ultimately supporting photosynthesis and plant productivity. Conversely, the decrease at higher concentrations indicates that the extract shifts from being a stimulant to a stressor. The same bioactive compounds that elicit a positive response at low doses are suspected to trigger the production of stress hormones like ABA at high doses. The increase in ABA then induces stomatal closure as a defense mechanism to limit further uptake of allelopathic compounds and prevent excessive water loss (Gahir et al., 2021). The lack of a varietal effect can be attributed to the fact that the mechanisms of stomatal opening and closing are fundamental physiological processes at the species level, controlled by hormonal and environmental signals such as ABA, intracellular CO_2, and light (Hsu et al., 2021). Although Talam 2 and Hypoma 2 are distinct varieties, they belong to the same species (*A. hypogaea*). Therefore, the genetics and biochemistry regulating guard cell turgor and the response to stress signals are presumed to be highly similar.

Table 8. Effect of several concentrations of *A. spinosus* extract on the stomatal aperture width (μm) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	8.70	7.00	7.10	7.60
Talam 2 + 0.5%	8.73	7.60	8.30	8.21
Talam 2 + 1%	7.83	7.07	6.90	7.27
Talam 2 + 1.5%	6.30	6.57	6.50	6.46
Talam 2 + 2%	6.67	7.30	7.07	7.01
Hypoma 2 + 0%	7.30	6.30	7.20	6.93
Hypoma 2 + 0.5%	7.73	8.30	8.43	8.16
Hypoma 2 + 1%	6.77	7.37	7.20	7.11
Hypoma 2 + 1.5%	7.07	7.07	7.10	7.08
Hypoma 2 + 2%	6.97	7.17	6.17	6.77
Average	7.41	7.17	7.20	7.26

Stomatal aperture length

Stomatal aperture length refers to the size of the pore opening between guard cells, representing the dynamic, short-term regulatory mechanism that directly controls the rate of gas exchange. The complete dataset for stomatal aperture length across all treatment combinations is presented in Table 9. The mean of stomatal aperture length across all treatment was 15.28 μm, with values ranging from 14.14 to 17.36 μm. These data were subjected to a two-way ANOVA to examine the effects of variety, *A. spinosus* extract concentration, and their interaction. The results showed a statistically significant main effect of extract concentration on stomatal aperture length ($p<0.05$). In contrast, the main effect of variety ($p>0.05$) and the interaction between variety and concentration ($p>0.05$) were not statistically significant (Table 7). Given the significant main effect of concentration, a Duncan's post-hoc test was conducted. The test revealed that treatment with a 0.5% extract concentration resulted in the greatest stomatal aperture length (17.36±1.15 μm), which was significantly larger than the control (15.17±0.49 μm) and all other concentrations. Conversely, at concentrations of 1% to 2%, the stomatal aperture length decreased significantly, with the lowest value at the 2% concentration (14.14±0.88 μm), which was even lower than the control. This 14.4% increase at the 0.5% concentration indicates that at low doses, bioactive compounds in the extract, such as flavonoids and phenolic compounds, act as elicitors triggering a positive physiological response. According to (Kalaivani et al., 2023), plant-derived bioactive compounds can act as elicitors that not only stimulate growth but also induce physiological changes. This more optimal stomatal opening has the potential to enhance gas exchange, thereby promoting a higher photosynthetic rate (Driesen et al., 2020). This finding is consistent with the principle of hormesis, where exposure to mild stress stimulates an improvement in an organism's performance. Conversely, the reduction at higher concentrations demonstrates that at high doses, the *A. spinosus* extract shifts its role from a promoter to a stressor. Plants respond by closing their stomata as a defense mechanism to limit the entry of potentially damaging foreign compounds. Although protective, this stomatal closure can restrict photosynthesis and transpiration if sustained over the long term. The non-significant effect of variety, shohws that the regulatory mechanism for stomatal aperture length is conserved at the *A. hypogaea* species. Despite genetic differences, both varieties possess homologous signalling systems and physiological responses for regulating stomatal opening.

Stomatal conductance

Stomatal conductance directly measures the rate of gas diffusion through the stomata, quantifying their physical restriction on CO_2 and water vapor exchange between the leaf and the atmosphere. The complete dataset for stomatal conductance across all treatment combinations is presented in Table 10. The mean of stomatal conductance across all treatment was 0.2176 μmol m^{-2} s^{-1}, with values ranging from 0.1171 to 0.2872 μmol m^{-2} s^{-1}. These data were subjected to a two-way ANOVA to examine the effects of variety, *A.*

Table 9. Effect of several concentrations of *A. spinosus* extract on the stomatal aperture length (µm) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	16.13	15.00	15.17	15.43
Talam 2 + 0.5%	17.53	16.10	17.23	16.96
Talam 2 + 1%	15.27	14.37	13.93	14.52
Talam 2 + 1.5%	15.90	14.33	16.53	15.59
Talam 2 + 2%	14.43	13.90	15.67	14.67
Hypoma 2 + 0%	14.93	15.07	14.73	14.91
Hypoma 2 + 0.5%	16.03	18.73	18.53	17.77
Hypoma 2 + 1%	14.73	14.93	14.83	14.83
Hypoma 2 + 1.5%	14.17	14.73	14.67	14.52
Hypoma 2 + 2%	13.30	14.23	13.30	13.61
Average	15.24	15.14	15.46	15.28

spinosus extract concentration, and their interaction. The results showed a statistically significant main effect of extract concentration on stomatal conductance ($p<0.05$). In contrast, the main effect of variety ($p>0.05$) and the interaction between variety and concentration ($p>0.05$) were not statistically significant (Table 7). Given the significant effect of concentration, a Duncan's post-hoc test was conducted. The test indicated that the control treatment (0%) exhibited the lowest stomatal conductance value (0.1171±0.0798 µmol m^{-2} s^{-1}). Increasing the extract concentration led to a significant increase in stomatal conductance, with the highest value achieved at the 1% concentration (0.2872±0.0904 µmol m^{-2} s^{-1}) (Figure 8).

At higher concentrations (1.5% and 2%), stomatal conductance remained at a high level and was not significantly different from the value at 1%, despite a slight numerical decrease. This 145% increase at the 1% concentration indicates that the spiny amaranth extract acts as a potent elicitor in stimulating stomatal opening. The increase in stomatal conductance is consistent with the previous stomatal aperture measurements but provides more functional information regarding the stomata's capacity for gas exchange. Higher stomatal conductance indicates improved efficiency in transpiration and the diffusion of CO_2 into the leaves for photosynthesis (Wang et al., 2022). The bioactive compounds in the *A. spinosus* extract are suspected to influence ion regulation in the guard cells, specifically through the modulation of the H^+-ATPase proton pump, which governs guard cell turgor pressure. An initial increase in ROS functions as a beneficial warning signal, prompting adaptive responses in the plant. This is analogous to the mechanism where ABA induced H_2O_2 in guard cells activates Ca^{2+} channels, ultimately leading to stomatal

Table 10. Effect of several concentrations of *A. spinosus* extract on the stomatal conductance (µmol m^{-2} s^{-1}) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	0.0550	0.0549	0.2476	0.1192
Talam 2 + 0.5%	0.1221	0.2120	0.2295	0.1878
Talam 2 + 1%	0.2153	0.3568	0.3934	0.3218
Talam 2 + 1.5%	0.1368	0.2427	0.4554	0.2783
Talam 2 + 2%	0.2189	0.2291	0.4012	0.2831
Hypoma 2 + 0%	0.1084	0.0591	0.1778	0.1151
Hypoma 2 + 0.5%	0.2214	0.1191	0.2598	0.2001
Hypoma 2 + 1%	0.2564	0.1614	0.3402	0.2527
Hypoma 2 + 1.5%	0.2708	0.2313	0.0572	0.1864
Hypoma 2 + 2%	0.2212	0.1160	0.3588	0.2320
Average	0.1826	0.1782	0.2921	0.2176

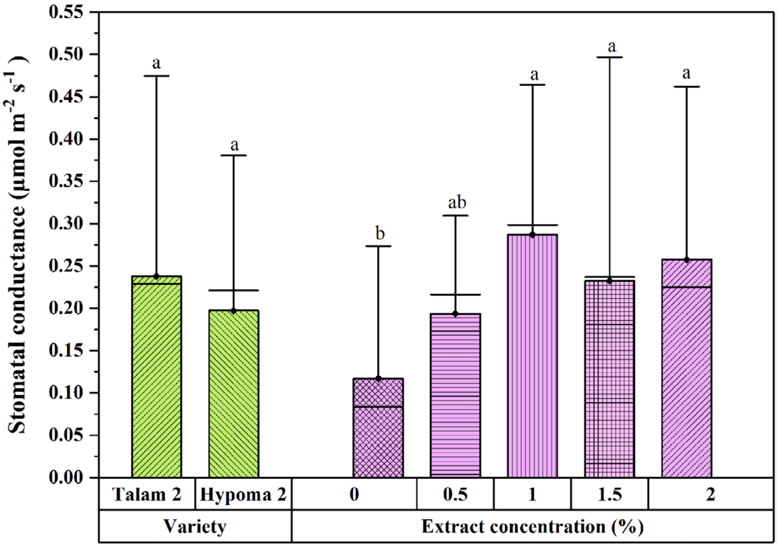

Figure 8. Stomatal conductance of peanut. Values with the same letter are not significantly different based on the Duncan's Multiple Range Test (α = 0.05)

closure (Hasanuzzaman et al., 2021). The sustained high level of stomatal conductance at concentrations up to 2% demonstrates that the plants continued to respond positively to the extract treatment within the tested range.

Transpiration rate

Transpiration rate quantifies the loss of water vapor from plant tissues, primarily via stomata. This process represents a critical component of the soil plant atmosphere continuum and serves as the driving force for the passive uptake of soil water and nutrients. The complete dataset for transpiration rate across all treatment combinations is presented in Table 11. The mean of transpiration rate across all treatment was 0.0206 µmol m^{-2} s^{-1}, with values ranging from 0.0106 to 0.0290 µmol m^{-2} s^{-1}. These data were subjected to a two-way ANOVA to examine the effects of variety, *A. spinosus* extract concentration, and their interaction. The results showed a statistically significant main effect of extract concentration on the transpiration rate ($p<0.05$). In contrast, the main effect of variety ($p>0.05$) and the interaction between variety and concentration ($p>0.05$) were not statistically significant (Table 7). Given the significant effect of concentration, a Duncan's post-hoc test was conducted. The test indicated that the control treatment (0%) exhibited the lowest transpiration rate (0.0106 ± 0.0074 µmol m^{-2} s^{-1}). Increasing the extract concentration led to a significantly higher transpiration rate, which peaked at the 1% concentration (0.0290 ± 0.0122 µmol m^{-2} s^{-1}) (Figure 9).

At higher concentrations (1.5% and 2%), the transpiration rate remained elevated and was not significantly different from the value at 1%, despite a slight numerical decrease. This 173% increase demonstrates that the spiny amaranth extract effectively influences stomatal regulation, directly impacting the transpiration process. The pattern observed in the transpiration rate was highly consistent with the previous stomatal conductance results, demonstrating a strong physiological correlation between these two parameters. The increase in stomatal conductance from 0.1172 to 0.2870 µmol m^{-2} s^{-1} (145%) aligned with the rise in transpiration rate from 0.0106 to 0.0290 µmol m^{-2} s^{-1} (173%). This relationship conforms to the plant physiology principle that the transpiration rate is directly influenced by stomatal conductance (Deushi et al., 2025). The mechanism of action of the bioactive compounds in the extract is suspected to involve the modulation of guard cell turgor pressure via the regulation of K^+ and anion ions. The elicitor compounds in the extract potentially interact with stomatal-regulating hormones like abscisic acid (ABA) or auxin, possibly by disrupting ABA signaling or biosynthesis, thereby reducing its inhibitory effect on stomatal opening. This increased stomatal aperture subsequently enhances the diffusion gradient of water vapor from the leaf to the atmosphere, a process known as transpiration. This finding aligns with the report by (Bajwa et al., 2020). that the exogenous application of allelochemicals extracted from plants can enhance physiological processes, including stomatal movement, membrane permeability,

Table 11. Effect of several concentrations of *A. spinosus* extract on the transpiration rate (μmol m^{-2} s^{-1}) of Talam 2 and Hypoma 2 peanut varieties

Treatment (Variety + *A. spinosus* extract concentration)	Block 1	Block 2	Block 3	Average
Talam 2 + 0%	0.0047	0.0049	0.0208	0.0101
Talam 2 + 0.5%	0.0141	0.0187	0.0168	0.0165
Talam 2 + 1%	0.0188	0.0380	0.0461	0.0343
Talam 2 + 1.5%	0.0135	0.0187	0.0448	0.0257
Talam 2 + 2%	0.0186	0.0190	0.0376	0.0251
Hypoma 2 + 0%	0.0095	0.0047	0.0188	0.0110
Hypoma 2 + 0.5%	0.0192	0.0094	0.0231	0.0172
Hypoma 2 + 1%	0.0239	0.0142	0.0331	0.0237
Hypoma 2 + 1.5%	0.0288	0.0189	0.0047	0.0175
Hypoma 2 + 2%	0.0239	0.0094	0.0416	0.0250
Average	0.0175	0.0156	0.0287	0.0206

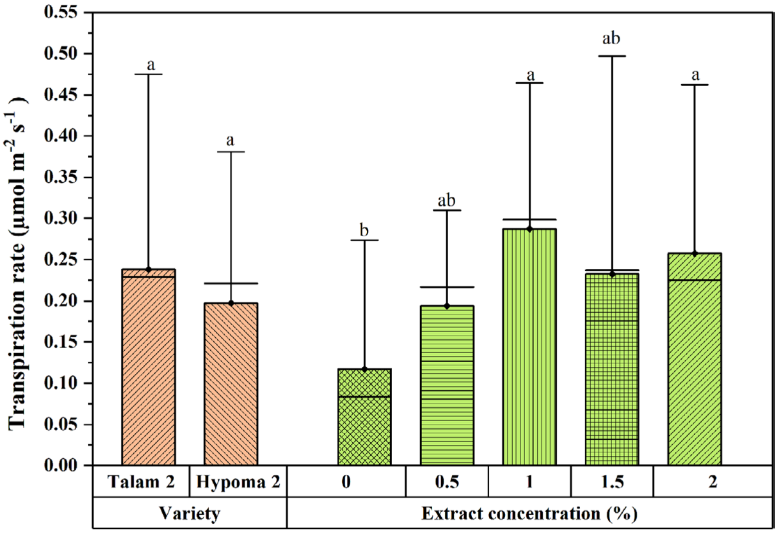

Figure 9. Transpiration rate of peanut. Values with the same letter are not significantly different based on the Duncan's Multiple Range Test and (α = 0.05)

and hormonal balance. The lack of a significant varietal effect, likely attributed to conserved genetic mechanisms governing fundamental physiological processes like stomatal function across the tested peanut varieties.

CONCLUSIONS

The extract of *A. spinosus* demonstrably induces hormetic effects on the physiological performance of peanut plants. A clear biphasic response was observed, where low to intermediate concentrations (0.5–1%) consistently stimulated physiological activity, whereas higher concentrations (1.5–2%) were predominantly inhibitory. The manifestation of this hormesis was highly parameter-dependent. Dynamic processes, including stomatal aperture dimensions, stomatal conductance, and transpiration rate, exhibited the most pronounced and consistent stimulation, peaking within the 0.5–1% concentration range. In contrast, constitutive structural traits such as stomatal density and leaf greenness remained largely unaffected, indicating that the extract's primary influence is on functional physiology rather than permanent anatomical structures. A significant genotypic influence was evident in the response of photosynthetic pigments. The Talam 2 variety displayed a well-defined classical hormetic response, with clear optimal concentrations for chlorophyll biosynthesis. The Hypoma 2 variety, however, showed a more variable and less pronounced stimulatory pattern. Conversely, stomatal regulatory responses were consistent across both varieties, suggesting a conserved mechanism of action for the extract's bioactive compounds on stomatal function. In summary, this study establishes that *A. spinosus* extract functions as a potent plant biostimulant at low concentrations, with its efficacy being contingent upon both the specific physiological trait targeted and the genetic background of the crop variety.

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