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Research Article

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Investigating the Growth-Promoting Potential of Bryophyte Extracts from *Bryum argenteum*, *Fissidens dubius*, and *Plagiochasma appendiculatum* on Wheat (*Triticum aestivum* L.): Implications for Sustainable Agricultural Practices

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ABSTRACT
This study investigates bryophyte extracts as sustainable alternatives to chemical fertilizers for wheat (*Triticum aestivum* L.), a vital crop facing rising demand and environmental challenges such as soil degradation and water pollution. Extracts from three bryophyte species—Bryum argenteum, Fissidens dubius, and Plagiochasma appendiculatum—collected in Kotli, Azad Kashmir, in 2024, were tested. Ethanol, methanol, and aqueous extracts were applied at concentrations of 20ml, 30ml, and 40ml to wheat seeds in petri plates, which were incubated for 14 days at 25±2°C with 10–12 hours of light daily. Although germination rates showed no significant differences ($p>0.05$), the 40 ml aqueous extracts significantly boosted shoot length (up to 40% increase) and root length (up to 36.58% increase) across species compared to controls. Methanolic extracts (40ml) excelled in biomass, enhancing fresh weight (up to 21.32% increase) and dry weight (up to 22.92% increase), while moisture content peaked with methanolic extracts (up to 28.72% increase). These findings highlight bryophyte extracts as eco-friendly growth enhancers, reducing reliance on synthetic fertilizers and their environmental harm. Further exploration of active compounds and field trials is recommended.
Keywords: *Bryum argenteum*, *Fissidens dubius*, *Plagiochasma appendiculatum*, wheat germination, plant growth regulators, bryophyte extracts, *T. aestivum*

INTRODUCTION

Wheat (*Triticum aestivum* L.), a member of the Poaceae family, is a vital cereal crop underpinning human nutrition and livestock feed worldwide. Originating during the Neolithic Revolution around 10,000 years ago, wheat has evolved into a staple cultivated across diverse agroecosystems (Dubcovsky & Dvorak, 2007). It occupies approximately 9 million hectares globally, providing 37% of dietary protein and energy for millions (Khan et al., 2008). In Pakistan, wheat ranks among the top agricultural commodities, positioning the country as the ninth-largest producer by cultivated area and fifth by yield (Anonymous, 2007). Its versatility—from bread and noodles to livestock fodder—combined with nutritional benefits (dietary fibers, antioxidants, and minerals), underscores its economic and cultural significance (Shewry, 2000).

However, wheat production faces challenges from biotic (pests, pathogens) and abiotic (drought, salinity) stressors, compounded by declining soil fertility due to excessive chemical fertilizer use (Falls & Siegel, 2005). These synthetic inputs, while boosting yields, contribute to environmental degradation, including soil pollution, eutrophication, and heavy metal accumulation in the food chain (Kohler et al., 2010). Consequently, there is a pressing need for sustainable alternatives that enhance crop performance without compromising ecological health.

Bryophytes—non-vascular plants encompassing mosses, liverworts, and hornworts—offer a promising solution. With over 24,000 species globally and 2,000 in South Asia, bryophytes are ecologically significant yet underutilized in agriculture (Sabovljević et al., 2016). They lack true roots but possess rhizoids for anchorage and water absorption, thriving in diverse habitats from deserts to wetlands (Vashishta, 1963). Historically, bryophytes have been valued in ethnobotany for medicinal and ecological roles, such as nitrogen fixation and soil stabilization (Harper & Marble, 1988). Recent studies highlight their production of bioactive compounds—lipids, terpenoids, and phytohormones—that exhibit antibacterial, antifungal, and growth-regulating properties (Harris, 2008; Huneck & Meinunger, 1990). This study leverages these attributes to explore bryophyte extracts as natural growth promoters for wheat.

Study Species

Bryum argenteum: A cosmopolitan moss known for its desiccation tolerance and adaptability to urban and desert environments (Hui et al., 2013). It contributes to soil crusts and nutrient cycling (Zhang et al., 2007).

Fissidens dubius: A moss with distinct morphological traits, noted for its ecological roles in microhabitat formation and nutrient dynamics (Brown & Smith, 2014).

Plagiochasma appendiculatum: A liverwort with unique reproductive structures, recognized for its potential in biodiversity and soil fertility enhancement (Smith et al., 2014).

The objectives of the present study were: to prepare and characterize ethanolic, methanolic, and aqueous extracts from *B. argenteum, F. dubius,* and *P. appendiculatum* and to evaluate their effects on wheat seed germination and seedling growth parameters.

MATERIALS AND METHODS

Plant Material Collection

Bryophyte samples were collected during field expeditions in Kotli, Azad Kashmir (33°30' N, 73°53' E), a region with a subtropical climate conducive to bryophyte diversity. *Bryum argenteum* was harvested from rocky outcrops, *Fissidens dubius* from shaded forest floors, and *Plagiochasma appendiculatum* from moist stream banks. Collections occurred in spring 2024 to ensure optimal plant vitality. Specimens were authenticated by Dr. Asim Shahzad and Dr. Javeed Hussain, cross-referenced with Flora of Pakistan (www.tropicos.org). Wheat seeds (*Triticum aestivum L*., cultivar 'Pakistan-13') were procured from NARC, Islamabad, ensuring uniformity and viability.

Extract Preparation

Fresh thalli were thoroughly washed with double-distilled water to remove debris, air-dried at room temperature (25 ± 2°C) for 48 hours, and mechanically chopped into 1–2 mm fragments using a sterile blade. For each species, 50 g of processed material was soaked in 200 ml of solvent (95% ethanol, 99% methanol, or double-distilled water) in 500 ml Erlenmeyer flasks. Extraction proceeded for seven days with periodic shaking (twice daily) to enhance solute diffusion. Solutions were filtered through Whatman No. 1 filter paper under a laminar flow hood to maintain sterility, yielding clear filtrates. These were stored in airtight amber glass bottles, labelled by species and solvent, and refrigerated at 4°C to preserve bioactive compounds.

Seed Sterilization and Germination Setup

Wheat seeds were surface-sterilized to eliminate microbial contamination: soaked in 95% ethanol for 1 minute, treated with 10% Clorox (sodium hypochlorite) solution for 2–3 minutes, and rinsed five times with sterile distilled water. For germination, 10 seeds per replicate were placed on double-layered Whatman No. 1 filter paper in 90 mm petri plates. Each plate received 20 ml, 30 ml, or 40 ml of extract (ethanolic, methanolic, or aqueous) or distilled water (control). Plates were sealed with parafilm, incubated at 25 ± 2°C, and illuminated with a 40w fluorescent lamp (10–12 hours daily) to simulate natural light conditions.

Table1. Treatment application

Extract Used	Treatments	Application
Ethanol Extract	T-1	Control (distilled water)
	T-2	20 ml
	T-3	30 ml
	T-4	40 ml
Methanol Extract	T-1	Control (distilled water)
	T-2	20 ml
	T-3	30 ml
	T-4	40 ml
Aqueous Extract	T-1	Control (distilled water)
	T-2	20 ml
	T-3	30 ml
	T-4	40 ml

Experimental Design

The experiment employed a completely randomized design (CRD) with three replicates per treatment (12 treatments × 3 species × 3 replicates = 108 experimental units), given in Table 1. Treatments were:

Measurement of Growth Parameters

After 14 days, the following parameters were assessed:

Germination Percentage: Counted as the proportion of seeds with emerged radicles, calculated as:

$$Percentage\ Seed\ Germination = \frac{Number\ of\ Germinating\ Seeds}{Total\ Seeds\ Used} \times 100$$

Shoot Length: Measured from the seed base to the shoot apex in centimetres using a meter rod, averaging three randomly selected seedlings per plate.

Root Length: Measured from the seed base to the root tip, similarly averaged.

Fresh Weight: Determined immediately after harvest using a Sartorius electronic balance (precision: 0.001 g).

Dry Weight: Recorded after oven-drying seedlings at 70°C for 48 hours until constant weight (Agrawal, 1986).

Moisture Content: Calculated as:

$$\text{Moisture Content of Shoot} = \text{fresh weight of shoot} - \text{dry weight of shoot}$$

$$\text{Moisture Content of Root} = \text{fresh weight of root} - \text{dry weight of root}$$

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using MiniTab and Microsoft Excel to detect significant differences ($p<0.05$) among treatments. Means and standard deviations were computed for each parameter, and Tukey's HSD test was applied for post-hoc comparisons. Results were visualized using bar graphs with error bars to illustrate variability.

RESULTS AND DISCUSSION

Germination Percentage

Germination was uniform across all treatments, averaging 90–95% after 14 days, with no significant differences (p>0.05) among ethanolic, methanolic, and aqueous extracts of *B. argenteum*, *F. dubius*, and *P. appendiculatum* (Fig. 1 a–c). Control plates showed comparable germination (92 ± 3%), suggesting that extracts neither inhibited nor uniquely enhanced this parameter.

Shoot Length

Shoot length increased with extract concentration, peaking at 40 ml (p<0.05):

B. argenteum: Aqueous (18.43 ± 1.02 cm, 33.87% increase over control), methanolic (16.92 ± 0.89 cm, 16.92% increase), ethanolic (15.94 ± 0.76 cm, 15.94% increase). *F. dubius*: Aqueous (18.22 ± 1.02 cm, 30.16% increase), methanolic (16.67 ± 0.85 cm, 16.67% increase), ethanolic (14.49 ± 0.71 cm, 14.49% increase). *P. appendiculatum*: Aqueous (18.67 ± 1.54 cm, 40% increase), methanolic (15.63 ± 0.82 cm, 15.63% increase), ethanolic (9.30 ± 0.65 cm, 9.30% increase). Aqueous extracts consistently outperformed others by 13.5–30.5% (Fig. 2 a–c).

Root Length

Root length followed a similar trend:

B. argenteum: Aqueous (27.67 ± 1.53 cm, 30.71% increase), methanolic (16.03 ± 0.94 cm, 16.03% increase), ethanolic (16.80 ± 0.88 cm, 16.80% increase). *F. dubius*: Aqueous (27.33 ± 1.53 cm, 27.14% increase), methanolic (17.22 ± 0.91 cm, 16.53% increase), ethanolic (17.55 ± 0.87 cm, 15.33% increase). *P. appendiculatum*: Aqueous (28.00 ± 2.31 cm, 36.58% increase), methanolic (16.55 ± 0.90 cm, 15.49% increase), ethanolic (17.24 ± 0.85 cm, 10.06% increase). Aqueous extracts led by 10.6–26.5% (p<0.05) (Fig. 3 a–c).

Fresh Weight

Fresh weight peaked with 40 ml of methanolic extracts:

B. argenteum: Methanolic (1.59 ± 0.02 g, 20.40% increase), aqueous (1.52 ± 0.03 g, 17.12% increase), ethanolic (1.40 ± 0.02 g, 13.65% increase). *F. dubius*: Methanolic (1.59 ± 0.02 g, 20.40% increase), aqueous (1.52 ± 0.03 g, 17.12% increase), ethanolic (1.40 ± 0.02 g, 13.65% increase). *P. appendiculatum*: Methanolic (1.59 ± 0.03 g, 21.32% increase), aqueous (1.54 ± 0.03 g, 18.70% increase), ethanolic (1.42 ± 0.02 g, 15.77% increase). Methanolic extracts surpassed others by 2.55–6.67% (p<0.05) (Fig. 4a–c).

Dry Weight

Dry weight results mirrored fresh weight trends:

B. argenteum: Methanolic (0.79 ± 0.01 g, 22.92% increase), aqueous (0.76 ± 0.02 g, 18.68% increase), ethanolic (0.67 ± 0.01 g, 7.73% increase). *F. dubius*: Methanolic (0.79 ± 0.04 g, 22.92% increase), aqueous (0.76 ± 0.02 g, 18.68% increase), ethanolic (0.67 ± 0.01 g, 7.73% increase). *P. appendiculatum*: Aqueous (0.74 ± 0.07 g, 15.17% increase), methanolic (0.73 ± 0.03 g, 13.55% increase), ethanolic (0.68 ± 0.02 g, 11.00% increase). Solvent effects were significant (p<0.05) (Fig. 5 a–c).

Moisture Content

Moisture content varied:

B. argenteum: Ethanolic (0.81 ± 0.02 g, 19.00% increase), methanolic (0.80 ± 0.02 g, 18.06% increase), aqueous (0.77 ± 0.02 g, 15.62% increase). *F. dubius*: Methanolic (0.81 ± 0.06 g, 27.22% increase), aqueous (0.78 ± 0.03 g, 23.95% increase), ethanolic (0.73 ± 0.02 g, 17.86% increase). *P. appendiculatum*: Methanolic (0.81 ± 0.04 g, 28.72% increase), aqueous (0.79 ± 0.03 g, 21.90% increase), ethanolic (0.74 ± 0.02 g, 20.39% increase). Methanolic extracts often led (p<0.05) (Fig. 6 a–c).

The consistent germination rates (90–95%) across all bryophyte extract treatments, with no significant differences from controls (p >0.05), suggest that *B. argenteum*, *F. dubius*, and *P. appendiculatum* extracts are non-toxic and supportive of wheat seed viability. This aligns with Wu et al. (2015), who found that natural extracts often maintain baseline germination by providing essential nutrients or hormones without allelopathic inhibition. Bryophytes may release cytokinins or gibberellins, known to initiate germination by breaking seed dormancy (Sabovljević et al., 2010). The lack of variation across solvents (ethanolic, methanolic, aqueous) indicates that bioactive compounds are effectively extracted regardless of polarity, a finding consistent with Zhou et al. (1998), who reported 20–70% germination increases with moss extracts on various crops. However, the absence of a pronounced enhancement over controls suggests that germination may be less sensitive to bryophyte influence than subsequent growth stages, warranting further investigation into concentration thresholds.

The significant enhancement of shoot and root length by 40 ml aqueous extracts (*P. appendiculatum*: 40% and 36.58% increases, p<0.05) highlights a solvent-specific efficacy likely tied to polar phytohormones such as auxins and gibberellins, which regulate cell division and elongation (Huneck & Meinunger, 1990). Aqueous extracts outperformed methanolic and ethanolic counterparts by 10.6–30.5%, mirroring Kramski et al. (2023), who attributed wheat growth to water-soluble fungal metabolites. For instance, *B. argenteum*'s aqueous extract yielded a shoot length of 18.43 ± 1.02 cm, a 33.87% increase, possibly due to its desiccation-tolerant physiology concentrating growth factors (Hui et al., 2013). *P. appendiculatum*'s superior performance may reflect liverwort-specific compounds, such as phenylpropanoids, absent in mosses (Smith et al., 2014). This differential response suggests that solvent polarity influences the extraction of active constituents. Aqueous solutions likely dissolve hydrophilic molecules critical for structural development, while organic solvents (ethanol, methanol) may extract lipophilic compounds less effective for elongation. Compared to Sundus and Sundus (2022), who used mineral fertilizers to achieve similar shoot growth, bryophyte extracts offer an organic alternative with reduced ecological risk, aligning with sustainable agriculture principles (Godlewska et al., 2021).

Methanolic extracts' dominance in fresh weight (*B. argenteum*: 1.59 ± 0.02 g, 20.40% increase) and dry weight (0.79 ± 0.01 g, 22.92% increase) points to their efficiency in extracting non-polar metabolites—terpenoids, lipids, or phenolics—that enhance cellular mass and metabolic activity (Alam et al., 2015). This contrasts with aqueous extracts' focus on structural growth, suggesting a complementary role for solvent types. For *F. dubius*, methanolic extracts mirrored *B. argenteum*'s biomass gains, while *P. appendiculatum* showed a slight shift, with aqueous extracts leading in dry weight (0.74 ± 0.07 g), possibly due to its thalloid structure retaining more water-soluble biomass precursors (Smith et al., 2014).

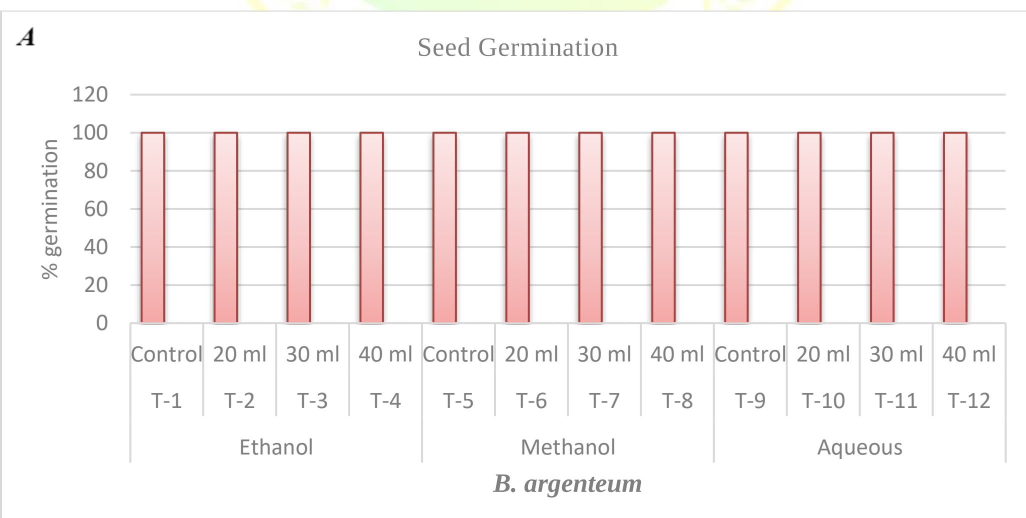
These results diverge from chemical fertilizer studies, where biomass increases often correlate with nitrogen or phosphorus uptake (Falls & Siegel, 2005). Bryophyte extracts, lacking such macronutrients, likely act via secondary metabolites, offering a low-input alternative. The 2.55–6.67% advantage of methanolic over other extracts ($p<0.05$) underscores the importance of solvent optimization, a factor underexplored in prior bryophyte research.

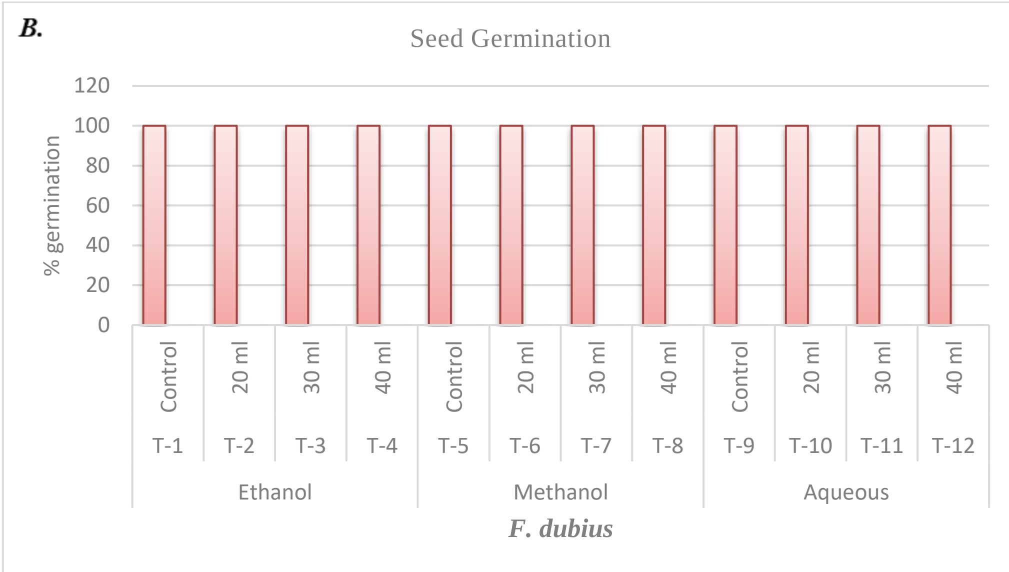
Moisture content's variability—methanolic extracts leading for *F. dubius* (0.81 ± 0.06 g, 27.22% increase) and *P. appendiculatum* (0.81 ± 0.04 g, 28.72% increase), but ethanolic for *B. argenteum* (0.81 ± 0.02 g, 19.00% increase)—suggests complex interactions between solvent properties and seedling physiology. Methanol's ability to extract organic osmolytes may enhance water retention, while ethanol's intermediate polarity could balance hydration and solute uptake (Alam et al., 2015). This variability deviates from typical fertilizer studies, where moisture is uniform, indicating bryophyte extracts influence cellular water dynamics uniquely, possibly via osmotic regulation or membrane stabilization (Sabovljević et al., 2016).

Bryophytes' ecological roles—soil stabilization (Conard, 1935), nitrogen fixation (Harper & Marble, 1988), and bio-indication (Taoda, 1973)—parallel their agricultural potential. *B. argenteum*'s desert adaptability (Zhang et al., 2007) and *P. appendiculatum*'s moisture retention (Smith et al., 2014) suggest species-specific advantages transferable to farming systems. Unlike synthetic PGRs, which disrupt soil microbiota (Kohler et al., 2010), bryophyte extracts could enhance microbial diversity, supporting long-term soil health. Their natural origin aligns with organic farming trends, reducing reliance on fossil fuel-derived inputs (Godlewska et al., 2021).

Compared to plant extract studies (Mamun & Shahjahan, 2011, on indigenous plants), bryophytes offer a novel niche due to their non-vascular physiology and biochemical diversity. While fungal extracts (Kramski et al., 2023) and seaweed extracts (Chandini et al., 2008) enhance growth, bryophytes' desiccation tolerance and ecological resilience provide unique advantages in stress-prone regions like Pakistan. This study's focus on wheat, a globally critical crop, amplifies its relevance, bridging bryology and agronomy.

The Petri plate design limits extrapolation to field conditions, where soil interactions and climate variability could alter outcomes. The lack of phytochemical profiling hinders identification of active compounds, a gap noted in prior bryophyte research (Sabovljević et al., 2016). Variability in moisture content suggests potential inconsistencies in extract preparation or seedling response, necessitating standardized protocols. Additionally, the short 14-day duration may not capture long-term growth effects, such as yield or grain quality.





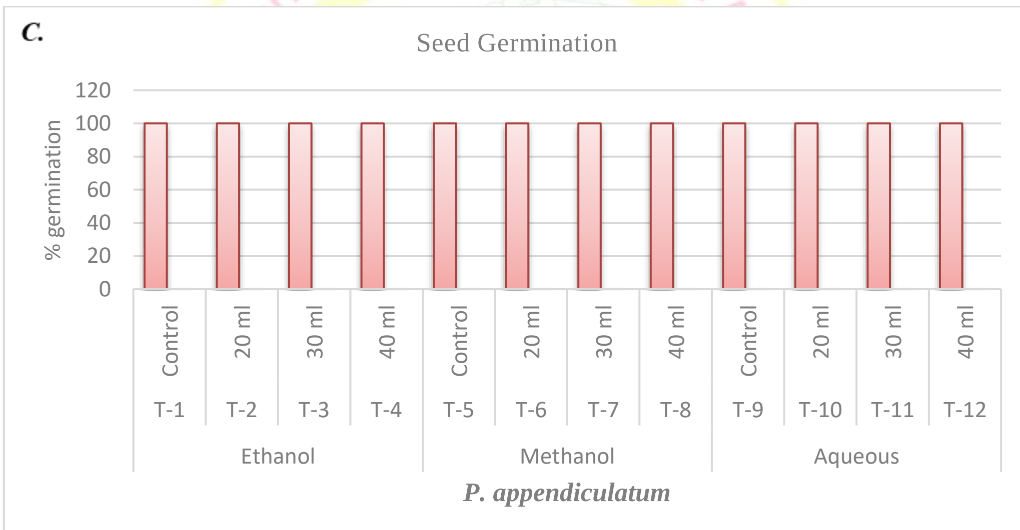


Fig. 1 (a-c), Percentage germination of wheat in response to different concentrations. of different extracts of (a) *B. argenteum (b) F. dubius (c) P. appendiculatum*

All treatments sharing the common letter with similar bar patterns are similar; otherwise vary significantly at p<0.05.

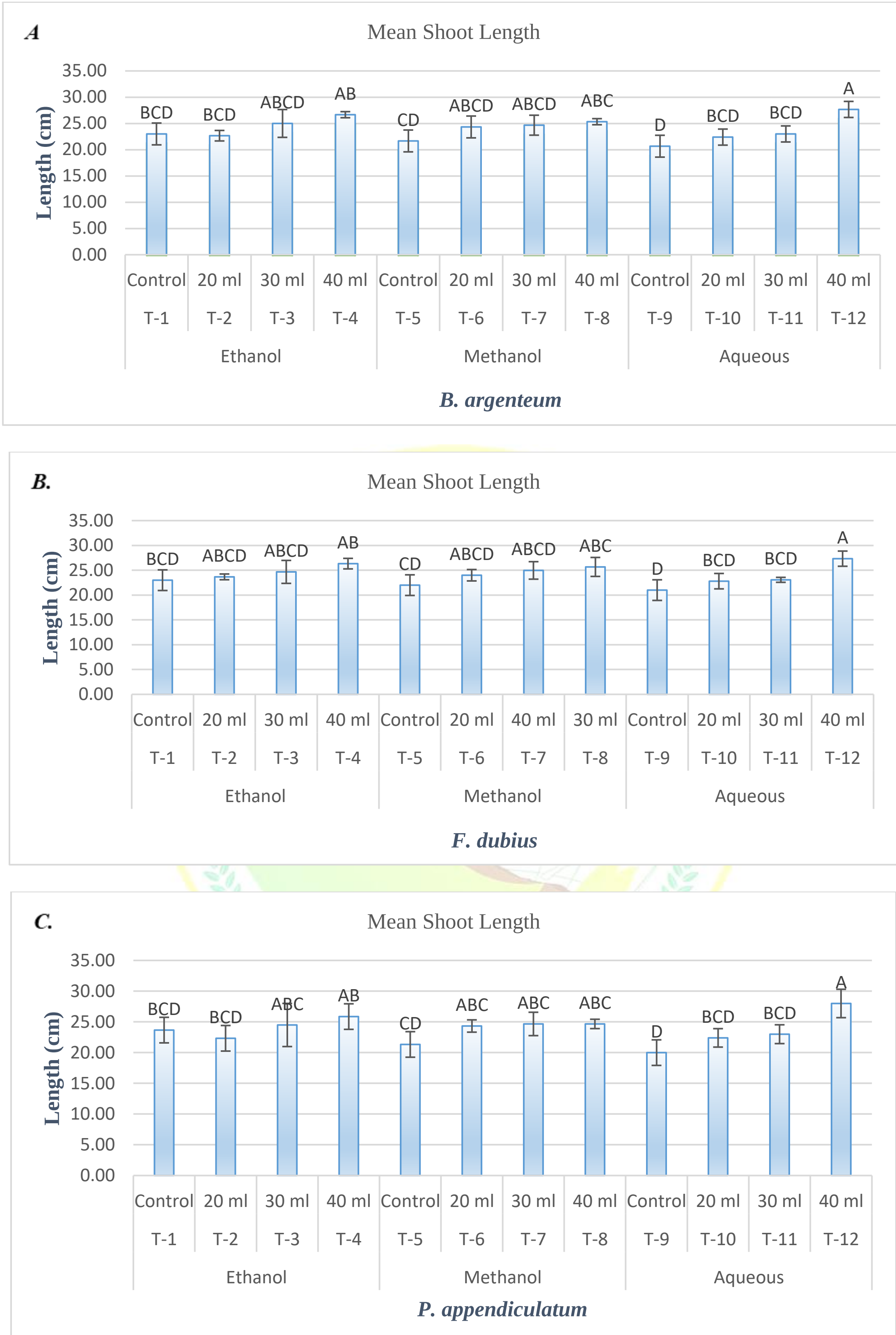
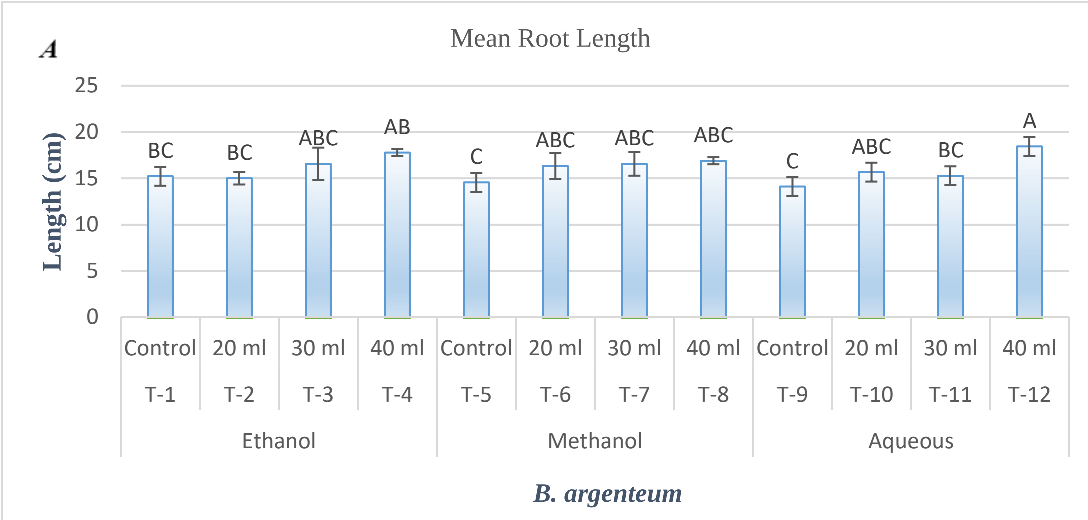
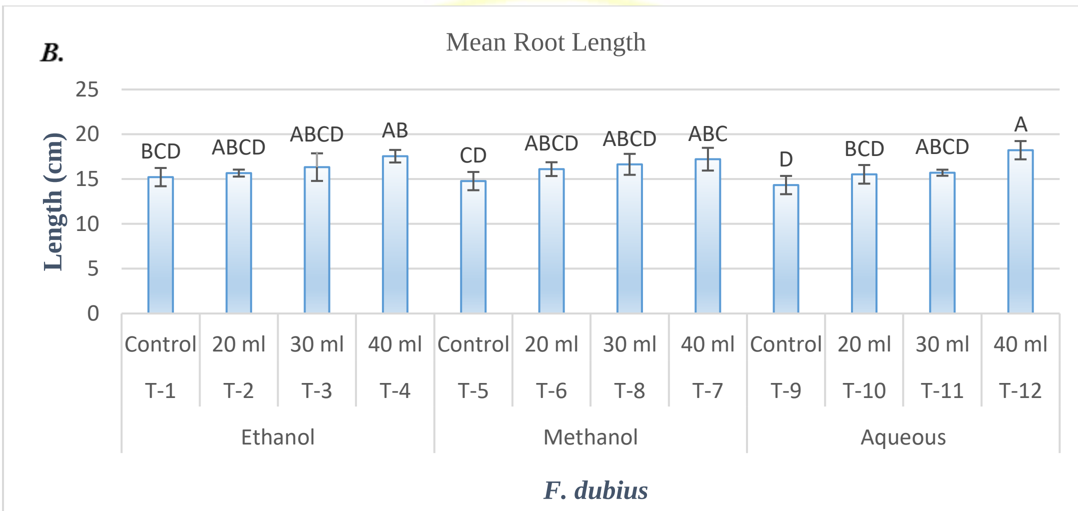


Fig. 2 (a-c), Average shoot length of wheat in response to different conc. of different of extracts of (a) *B. argenteum (b) F. dubius (c) P. appendiculatum*.

All treatments sharing common letter with similar bar pattern are similar otherwise vary significantly at $p<0.05$.





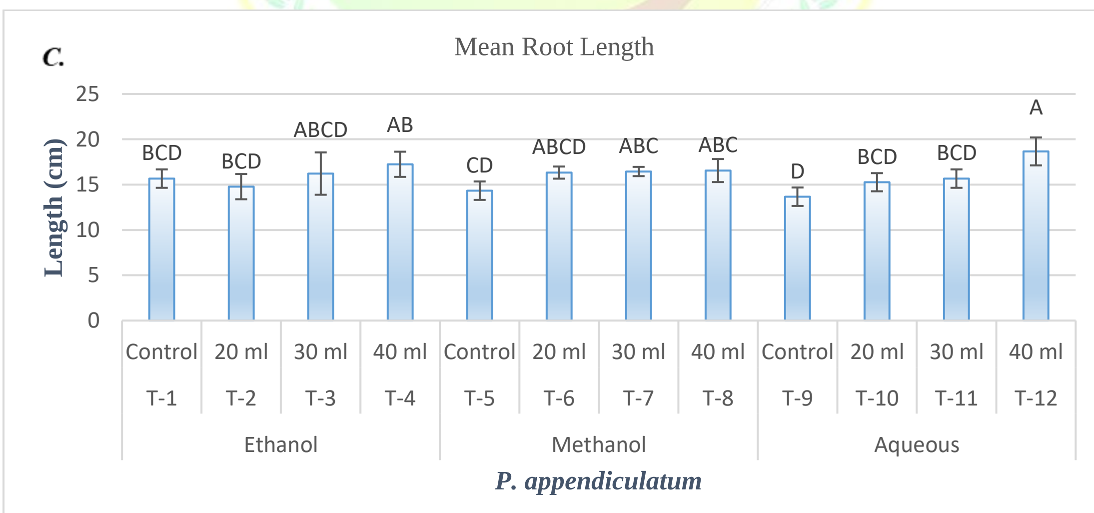


Fig. 3 (a-c), Average root length of wheat in response to different concentrations. of different extracts of (a) *B. argenteum (b) F. dubius (c) P. appendiculatum*.

All treatments sharing the common letter with a similar bar pattern are similar; otherwise vary significantly at $p<0.05$

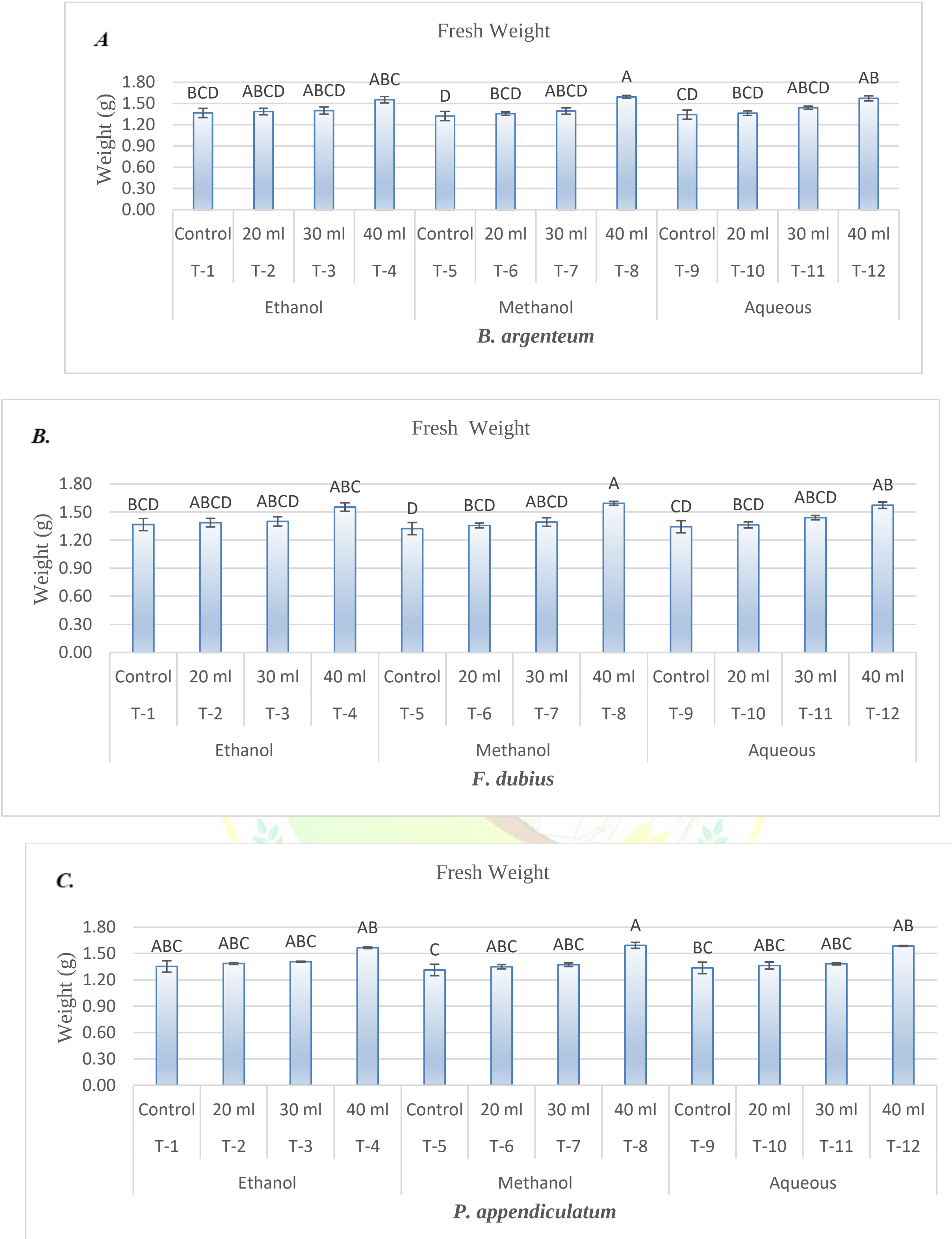


Fig. 4 (a-c), Average fresh weight of wheat in response to different concentrations. of different extracts of (a) *B. argenteum (b) F. dubius (c) P. appendiculatum*.

All treatments sharing common letter with similar bar pattern are similar; otherwise vary significantly at $p<0.05$.

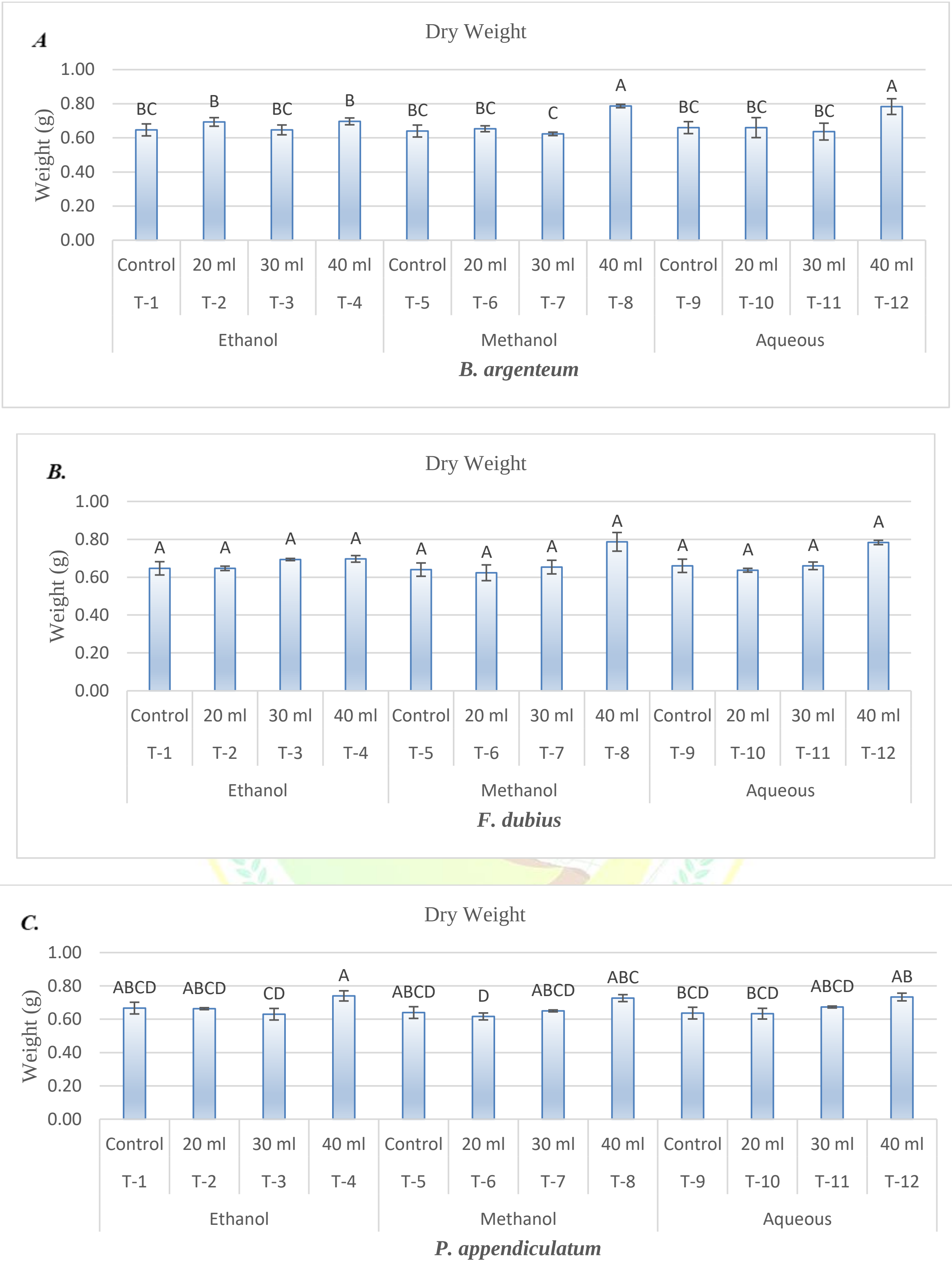


Fig. 5 (a-c), Average dry weight of wheat in response to different concentrations. of different extracts of (a) *B. argenteum (b) F. dubius (c) P. appendiculatum*,

All treatments sharing the common letter with a similar bar pattern are similar; otherwise vary significantly at $p<0.05$.

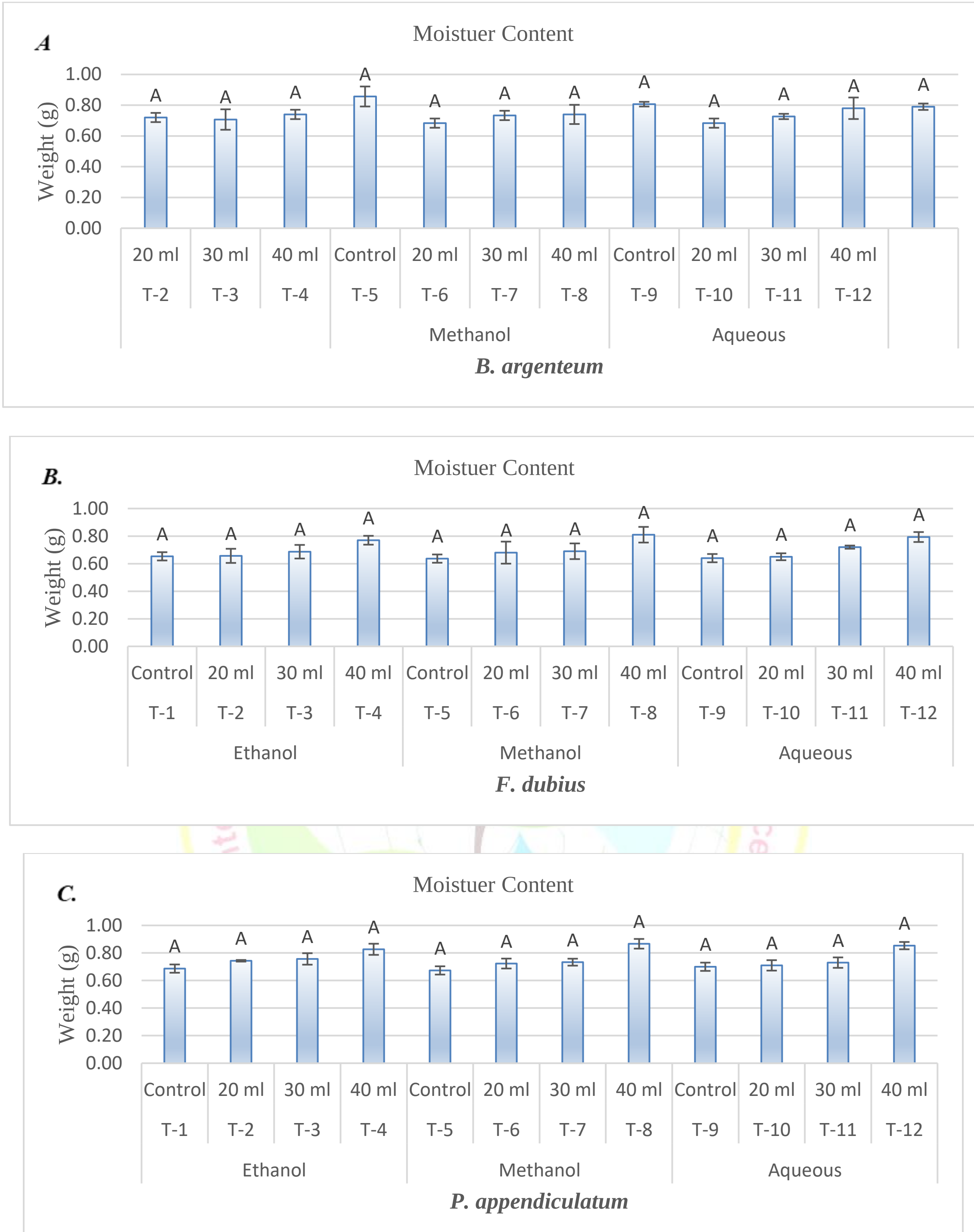


Fig. 6 (a-c), Average moisture content of wheat in response to different concentrations. of different extracts of (a) *B. argenteum (b) F. dubius (c) P. appendiculatum*.

All treatments sharing the common letter with similar bar patterns are similar; otherwise vary significantly at $p<0.05$.

CONCLUSION

This research establishes *Bryum argenteum*, *Fissidens dubius*, and *Plagiochasma appendiculatum* extracts as effective growth promoters for wheat (*T. aestivum* L.). Aqueous extracts (40 ml) excelled in shoot (up to 40% increase) and root length (up to 36.58% increase), while methanolic extracts maximized biomass (fresh weight up to 21.32%, dry weight up to 22.92%). These findings position bryophytes as sustainable alternatives to synthetic fertilizers, potentially reducing soil pollution and supporting ecological agriculture. Solvent choice and concentration critically influence efficacy, with aqueous extracts broadly effective for structural growth and methanolic for biomass. This study lays a foundation for leveraging bryophytes in modern farming, bridging their ecological significance with practical utility.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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