ASSESSMENT OF BIO-STIMULATORY EFFECTS OF A BRYOPHYTE SPECIES ON GERMINATION AND GROWTH OF *ZEA MAYS* L. AND *VIGNA UNGUICULATA* (L.) WALP.

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ABSTRACT

Aqueous extracts and litters of the moss *Archidium ohioense* were prepared. This was with a view to investigating the bio-stimulatory potential of the moss plant on the germination of *Zea mays* and *Vigna unguiculata* seeds. The moss sample was collected on the Obafemi Awolowo University campus, Ile-Ife, Nigeria. The collected sample was air dried at an ambient temperature in the laboratory. Four different extract concentrations of the moss plant were prepared by soaking 20 g and 40 g of the air-dried sample separately in 400 ml distilled water, each for 24 and 48 hours. Crushed litter of the air-dried moss sample (2.5 g) was also weighed into sterile Petri-dishes. The various extract concentrations and the crushed litter were experimented for their effects on the germination of *Z. mays* and *V. unguiculata* seeds following a method that was based on those earlier reported with a slight modification. The results of the experiment showed no inhibitory effect on the germination of the experimented seeds but enhanced the germination of seeds of both *Z. mays* and *V. unguiculata* and promoted their growth. The study concluded that the aqueous extract of *A. ohioense* enhanced the germination and growth of seeds both in *Z. mays* and *V. unguiculata*, and can be considered as a safer alternative to synthetic plant growth promoters.

Keywords: *Archidium*, bio-stimulant, extract, germination, litter, seeds

Introduction

The term bryophyte is the common name for mosses, liverworts and hornworts, which are non-vascular terrestrial plants with a haplo-diplontic life cycle in which the gametophytic phase is dominant (Wickett *et al.,* 2014; Morris *et al.,* 2018). This group of plants are generally seen as small plants confined to humid habitats, avoiding exposure to direct sunlight. Nevertheless, they are the second highest assemblages among the land plants after the angiosperms, but they are less known because of their sizes which are generally small (Chandra *et al.,* 2017). In contrast to vascular plants, bryophytes rarely grow as single stems but in groups which form turfs, cushions, wefts or other growth forms (Magdefrau, 1982). Their survival capacity is enormous as they survive under wide variety of environmental conditions and form strong part of the ecosystem where they grow (Zechmeister *et al*., 2003). They grow in forests, wetlands, deserts and cold regions (Nath & Asthana, 2005). They are considered the closest modern relatives of the ancestors to the earliest terrestrial plants linking the vascular plant to their algal ancestors (Kenrick & Crane, 1997). They play important roles in nutrient cycling, soil formation, micro habitat provision for other plants and animals, and promotion of seed germination (Jiang *et al.,* 2015).

Seed germination is the initiation of the first developmental phase in the lifecycle of higher plants which is then followed by the post germinative growth of the seedling (Rajjou *et al.*, 2012; Fatematuz *et al.*, 2023). It usually starts with the absorption of water, and the visible sign that germination is complete is usually the penetration of the structures surrounding the embryo by the radicle (Bewley, 1997). However, several studies in various plant communities and greenhouse experiments have demonstrated that germination and/or seedling emergence of vascular plants can be affected by bryophytes (Asakawa, 1990). The effect may be either positive or negative depending on the habitat and the species. The positive effects are generally explained by the moisture conditions favourable to germination that is provided by the bryophytes; and the negative ones by the low light intensity and allelopathy (Zamfir, 2000).

The term "allelopathy" indicates a complex effect exerted by one plant species (the donor) to another one (the acceptor), through the release into the environment of a number of chemical substances, termed allelochemicals (Carrubba *et al.,* 2020). These allelochemicals have an advantage to the plant that produces them by preventing the growth of other plant species that may compete with it for resources such as CO_2 and sunlight (Sharma *et al.*, 2009). The transmission of the allelochemicals can occur in several ways. They may be released directly and continuously by the living plants in the form of volatile compounds emitted into the atmosphere as root exudates into the soil, or as chemicals formed by the microbial degradation of plant residuals (Azim, 2008).

Allelopathy in bryophyte shapes ecosystem by influencing species composition of both vascular plants and other bryophytes (Whitehead *et al.*, 2018). Earlier, Huneck & Schreiber (1972) reported that allelopathic chemicals obtained from bryophytes possess growth regulatory activities. According to Asakawa *et al.* (1976), higher plants sometimes do not grow in places inhabited by certain bryophytes because some bryophytes produce allelopathic compounds. Bryophyte extracts exhibit dual effects on seedling germination depending on the species; some bryophytes have growth inhibiting properties, others have growth enhancing activities stimulated additional ivestigations with additional species (Frahm *et al.,* 2012). Application of different concentrations of bryophyte extracts may vary the response to enhancement promotion and inhibition. Thus, growth regulation activity depends on the concentration of extracts used as suggested by the results of Asakawa (1982). Negative effects of bryophytes can be interpreted as an indirect response to increase dominance of vascular plants caused by increase in canopy layer, shading and overloaded litter fall on ground vegetation (van Wijk *et al.*, 2003).

Several other investigations on bryophytes showed that allelopathy targets not only other bryophytes, but also the germination of seeds of vascular plants (van Tooren 1990, Basile *et al*., 2003, Löbel *et al.,* 2006, Soudzilovskaia *et al.,* 2011). Many of these studies appeared to be triggered by the search for plants that produce substances that could potentially be used as natural herbicides (Liu *et al*., 2014) or growth promoters. Such studies were typically undertaken by soaking bryophytes in water and subjecting the test seeds to the aqueous extracts (Whitehead *et al*., 2018). Hence, this study investigated the effects of aqueous extracts and litter of *Archidium ohioense* on the germination of *Zea mays* and *Vigna unguiculata*.

Experimental

Plant materials

The plant materials for this research work included a bryophyte species, cowpea seeds and maize seeds. Mature, healthy *Archidium ohioense* Schimp ex. C. Mull, (Plate 1) was collected from Hill 2, along Road 8, Obafemi Awolowo University Campus, Ile-Ife, Nigeria, within Latitude 7° 31' 17.6124" N and Longitude 4° 31' 42.298" E. The plant sample was carefully separated from dirt and extraneous materials, properly washed in stream of flowing tap water and air dried in the laboratory at the ambient temperature. Cowpea seeds (*Vigna unguiculata* (L.) Walp. variety SAMPEA 16, colour white) and maize seeds (*Zea mays* L., variety DT STR-Y, color yellow) were obtained from the Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife.

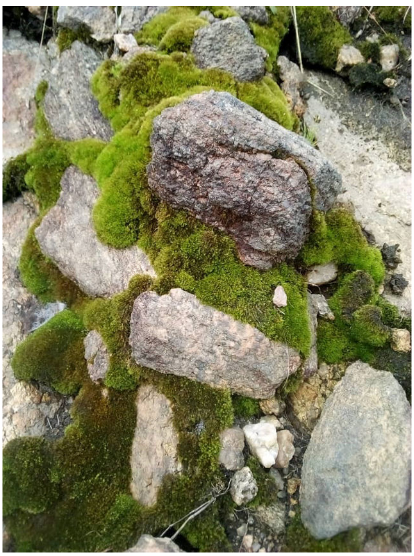

Plate 1: *Archidium ohioense* (arrow) on Hill 2 at the Obafemi Awolowo University Campus, Ile-Ife, Nigeria

Preparation of aqueous extracts of Archidium ohioense

From the air-dried *A. ohioense* sample 20 g and 40 g were separately soaked in 400 ml distilled water at the ambient temperature in the laboratory, each for both 24 hours and 48 hours, making four treatments. The suspensions were decanted into cheese cloth to remove the moss debris before filtration was carried out using Whatman No. 1 filter papers. The filtrates were stored in well labelled clean and covered glass bottles.

Germination experiment

Investigation of the effects of *A. ohioense* aqueous extracts and litters on seed germination and growth of *Zea mays* and *Vigna unguiculata* was carried out according to the methods of Ehsan *et al.* (2011) with little modification.

Effect of A. ohioense aqueous extract on seed germination

The inside of thoroughly washed sterile and dried Petri-dishes was lined with 3-folds of Whatman No. 1 filter papers. For each of the cowpea and maize, 10 seeds were placed on the filter papers in the Petri-dishes (Plates 2a, b, c, and d). For each treatment, 5 ml of the respective 24 hours and 48 hours prepared aqueous moss extracts of the 20 g / 400 ml and 40 g / 400 ml was added to the prepared Petri-dishes to moisten the filter papers and to provide moisture for the seeds to germinate. In the case of the control, 5 ml of distilled water was used. Both the treated and control were prepared in the fume chamber to avoid contamination. The Petri-dishes were incubated at 25°C in the laboratory for 72 hours. Germination, growth of plumule, radicle and seminal roots were noted after the expiration of 72 hours. Different Petri-dishes were prepared for the different extract treatments and there were five replicates for each of the experiments. The seedlings from the replicates of each treatment were then pulled together, and twenty (20) seedlings were randomly taken from each treatment for the determination of fresh weight and moisture contents. The numbers of seminal roots on the randomly selected seedlings were also noted for each treatment. The seedlings were dried at 65°C in the laboratory oven (Gallenkamp Size 1 incubator Model IH-150) for 72 hours, and the dry weights and moisture contents were determined.

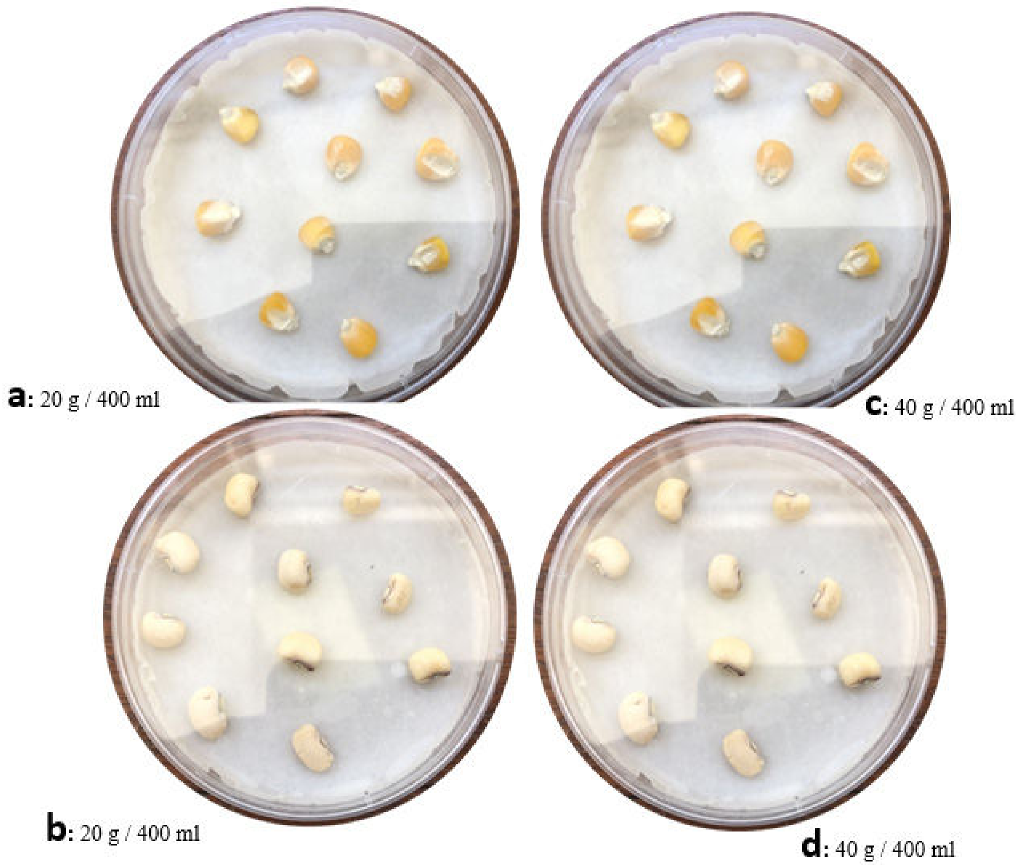

Plate 2: Prepared Petri-dishes for Archidium ohioense aqueous extract treatments (a) and (c) Zea mays (b) and (d) Vigna unguiculata

Effect of A. ohioense litters on seed germination

From crushed *A. ohioense* litter, 2.5 g was weighed into Petri-dishe. For each of *Z. mays* and *V. unguiculata* Ten (10) healthy seeds were placed on the litter in different Petri-dishes before 10 ml of distilled water was added (Plates 3a-b). In another set up, the 2.5 g moss litter in the Petri-dishes was topped with single sheet of Whatman No. 1 filter paper and the 10 seeds were placed on the filter paper (Plates 3c-d). Ten (10) ml of distilled water was added. There were five replicates for each of the experiments. After 72 hours of incubating the Petri-dishes at 25°C, germination, growth of plumule, radicle and seminal roots were noted. The seedlings from the replicates of each treatment were then pulled together, and Twenty (20) seedlings were randomly selected from each treatment for the determination of fresh weight and moisture contents. The numbers of seminal roots on the seedlings were also noted for each treatment. The seedlings were dried at 65°C in the laboratory oven (Gallenkamp Size 1 incubator Model IH-150) for 72 hours, and the dry weights and moisture contents were determined.

Statitical analysis

The data generated in this study were statistically analyzed with one way ANOVA using Statistical Analysis System (SAS) version 9.00 software. The value of $P < 0.05$ was considered statistically significant.

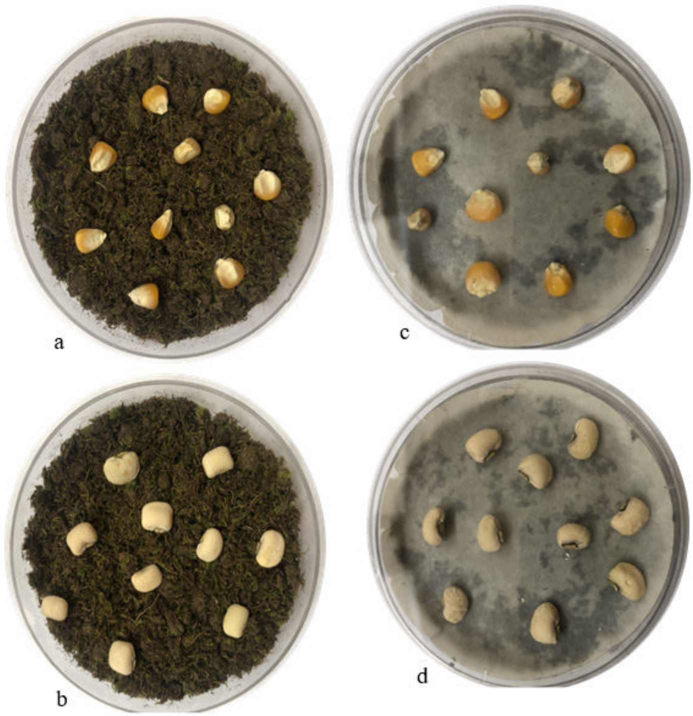

Plate 3: Petri-dishes set-up for testing the effects of *A. ohioense* litters on seeds germination: *Zea mays* (a and with filter paper: c); *Vigna unguiculata* (b and with filter paper: d)

Results

Effect of A. ohioense aqueous extracts on seeds germination

The results of the experiment on the effects of aqueous extracts of *A. ohioense* on germination of the seeds (*Zea mays* and *Vigna unguiculata*) showed that the aqueous extracts of *A. ohioense* had no inhibitory effects on the germination of the seeds. The summarized results of the observed and recorded effects of the extracts on the germination of *Z. mays* are presented in Tables 1 and 2; while Tables 3 and 4 showed the results of the effects of the extracts on the germination of *Vigna unguiculata.*

TABLE 1

Effect of aqueous extract of Archidium ohioense on germination, fresh and dry weights, and percentage moisture contents of Zea mays

Extracts	20 g/24 hrs	40 g/24 hrs	20 g/48 hrs	40 g/48 hrs	Control
Percentage Germination (%)	100	100	100	98	100
Fresh Weight (g)	7.48	8.06	7.99	7.74	7.39
Dry Weight (g)	3.95	3.76	4.40	4.34	3.99
Percentage Moisture (%)	47.19	53.35	44.93	43.93	46.01

TABLE 2

Effects of aqueous extracts of Archidium ohioense on plumule and radicle length, and number of seminal roots in Zea mays seedlings

Extracts	20 g/24 hrs	40 g/24 hrs	20 g/48 hrs	40 g/48 hrs	Control
Mean plumule length per seedling (mm)	9.8 ± 0.19^{b}	21.10 ± 0.14^{a}	8.00 ± 0.105^{b}	5.3 ± 0.12^{c}	5.9 ± 0.12^{c}
Mean radicle length per seedling (mm)	36.8 ± 0.23^{b}	48.30 ± 0.31^{a}	33.80 ± 0.21^{c}	30.0 ± 0.23^{d}	39.1 ± 0.28^{e}
Mean number of seminal roots/seedling	3.05 ± 0.37^{a}	3.70 ± 0.21^{a}	3.60 ± 0.26^{a}	3.10 ± 0.42^{a}	3.90 ± 0.32^{a}

Values are mean ± standard error (n = 20)

Means with the same superscript letters in a row are not significantly different (p ≤ 0.05).

TABLE 3

Effects of aqueous extracts of Archidium ohioense on Germination, fresh and dry weight, and percentage moisture contents of Vigna unguiculata

Extracts	20 g/24 hrs	40 g/24 hrs	20 g/48 hrs	40 g/48 hrs	Control
Percentage Germination (%)	100	100	92	100	100
Fresh Weight (g)	10.47	9.77	10.07	7.73	9.60
Dry Weighth (g)	3.35	3.77	3.69	2.85	3.85
Percentage Moisture (%)	68.00	61.41	63.36	63.13	59.90

TABLE 4

Effects of aqueous extracts of Archidium ohioense on plumule and radicle length, and number of seminal roots in Vigna unguiculata Seedlings

Extracts	20 g/24 hrs	40 g/24 hrs	20 g/48 hrs	40 g/48 hrs	Control
Mean plumule length per seedling (mm)	12.70 ± 0.24[a]	13.20 ± 0.19[a]	9.10 ± 0.11[b]	7.30 ± 0.10[c]	5.60 ± 0.12[d]
Mean radicle length per seedling (mm)	43.60 ± 0.39[a]	41.50 ± 0.41[b]	25.90 ± 0.18[c]	21.40 ± 0.26[d]	30.20 ± 0.31[e]
Mean Number of Seminal Roots/Seedling	8.15 ± 0.42[a]	5.75 ±0.54[b]	7.30 ± 0.94[a]	5.55 ± 0.87[b]	4.90 ± 0.78[b]

Values are mean ± standard error (n = 20)
Means with the same superscript letter in a row are not significantly different ($p \leq 0.05$).

Effect of A. ohioense litter on germination of Z. mays and V. Unguiculata seeds

A hundred percent seed germination was recorded in *Z. mays* in both the treatments and the control. The fresh weight and dry weight of the control were significantly ($p \leq 0.05$) higher than that of the other treatments. However, the percentage moisture content of the seedlings of the seeds planted directly in the moss litter was significantly ($p \leq 0.05$) higher compared to that of the seeds germinated on filter paper above *A. ohioense* litter and that of the control. The highest mean length of plumule (9.30 ± 0.12 mm) was recorded from the seedling of the seeds planted in moss litter and was significantly different ($p<0.05$) from that of the control but not significantly different from the plumule length of the seedlings from the seeds planted on filter paper above the moss litter. No statistically significant effect ($p<0.05$) was recorded for the mean length of radicle/ seedling and mean number of seminal roots/ seedlings of the treatments and that of control (Table 5).

Similarly, in *Vigna unguiculata* (Table 6), both the treatments and the control recorded 100% germination. The least fresh and dry weights were recorded in the control experiment while the highest fresh and dry weights were recorded in the treatments. The highest percentage moisture content was recorded from the seedling of the seeds planted in the moss litter. The highest mean plumule and radicle lengths per seedlings were recorded from the seedling of the seeds planted in moss litter and was statistically significantly different ($p < 0.05$) from that of the other treatment on filter paper above the moss litter, and the control. The highest mean number of seminal roots/seedlings was also recorded from the seedlings of the seeds planted in the moss litter.

TABLE 5

Effects of A. ohioense litter on germination of Z. mays

Treatments	Percentage Germination (%)	Fresh Weight (g)	Dry Weight (g)	Percentage Moisture (%)	Mean Length of Plumule/Seedling (mm)	Mean Length of Radicle/ Seedling (mm)	Mean Number of Seminal Roots/ Seedling
Seeds planted in Moss Litter	100	6.33	3.05	51.82	9.30 ±0.12[a]	34.50 ± 0.27[a]	3.70 ± 0.38[a]
Seeds planted on Filter Paper above Moss Litter	100	6.32	3.51	44.46	6.90 ± 0.10[ab]	32.70 ± 0.21[b]	3.30 ± 0.35[a]
Control	100	7.39	3.99	46.01	5.90 ± 0.12[b]	39.10 ± 0.28[c]	3.90 ± 0.32[a]

Plumule and radicle lengths and the number of seminal roots per seedling were expressed as means ± standard error (n = 20)

Means with the same letter in a column are not significantly different ($p < 0.05$).

TABLE 6

Effects of A. ohioense litter on germination of V. unguiculata seeds

Treatments	Percentage Germination (%)	Fresh Weight (g)	Dry Weight (g)	Percentage Moisture (%)	Mean Length of Plumule/ Seedling (mm)	Mean Length of Radicle/ Seedling (mm)	Mean Number of Seminal Roots/ Seedling
Seeds planted in Moss Litter	100	14.59	3.96	72.86	18.40 ± 0.28[a]	46.60 ± 0.48[a]	6.90 ± 1.68[a]
Seeds planted on Filter Paper above Moss Litter	100	11.39	4.21	63.04	7.10 ± 0.15[b]	30.90 ± 0.32[b]	3.30 ± 0.97[b]
Control	100	9.60	3.85	59.90	5.60 ± 0.12[b]	30.20 ± 0.31[b]	4.90 ± 0.78[c]

Plumule and radicle lengths and the number of seminal roots per seedling were expressed as means ± standard error (n = 20)Means with the same letter in a column are not significantly different ($p \leq 0.05$)

Discussion

The applications of bio-stimulants and phytohormones to promote germination of different plants have been reported. Bio-stimulants such as plant and animal products, humic substances, microorganisms, microbial inoculations, and several others have been reported to improve germination and root growth in different plants (Antonio-Medina *et al*., 2022).

Plant seeds germination and seedlings establishment are critical stages in plants development which determine both quality and quantity of crop yields (Subedi & Ma, 2005; Antonio-Medina, 2022). This study revealed that both the *Archidium ohioense* aqueous extracts and its litter enhanced the germination of the tested seeds of both *Zea mays* and *Vigna unguiculata* by promoting increase in the length of the plumules, radicles and the number of seminal roots of the seedlings. Germination of seeds started with the uptake of water, but, it may be influenced by various factors (Bewley, 1997; Bareke, 2018). Some plants interractions with other plants in their community could be harmful or beneficial (Hassan *et al.*, 2013). Some plants produce chemicals which affect other plants seed germinations and seedling growth (Alam & Islam, 2002).

Different concentrations of the moss *Porella platyphylla* extract inhibited the germination of cress and lettuce seeds while different concentrations of extracts of the moss *Brachythecium rutabulum* were reported to have no significant effects on the germination of cress seeds and lettuce seeds (Frahm *et al*., 2012). The observation on the effects of *A. ohioense* extracts on the germination and seedlings growth of *Z. mays* and *V. unguiculata* seeds in the present study agreed with the work of Sharma *et al.* (2009) who examined the growth regulatory activities of five mosses (*Brachythecium buchananii, Leucodon secundus, Timmiella anomala, Rhodobryum roseum* and *Plagiomnium integram*) on the germination behaviour of *Bidens biternata* seeds and recorded 100% germination.

Root growth and development are important aspects of seedling growth, which, if declines due to stress, drought or lack of nutrients may reduce the absorption and transportation of water and nutrients to the plant shoot, with a subsequent reduction in shoot growth (Steven, 2005), and ultimately limiting photosynthesis (Silvana, 1998). Plumule and radicle are parts of plant's embryo found inside the seed. During the germination of a seed, radicle is first developed through the micropyle of the seed, and it is the rudimentary root of the plant's embryo, developing into the root system which grows downward into the soil, absorbing water for further development of the root. Plumule emerges secondly from the seed and grows upwards away from the soil, developing into the shoot system which comprises the stem, the leaves and other floral parts (Lakna, 2017), of the plants.

Bryophytes are thought to be almost of no importance, but the occurrence of secondary metabolites of biologically active compounds in their tissues have increased their significance in relation to their application in several biological activities. This includes antifungal, antifeedant, growth regulatory and inhibitory effects (Pande *et al.*, 2004). The presence of these bioactive compounds in *A. ohioense* could have contributed to the influence of the extract on the germination and growth of the experimental seeds in this study which is also supported by Adedeji *et al*. (2012) and Edeoga & Eriata (2001).

In an experiment conducted by Huneck and Meinunger (1990), three different kinds of reactions were observed when seeds of cress were moistened with bryophytes extracts (52 mosses and 29 liverworts) and the lengths of the cress roots and shoots measured after five days at room temperature. It was reported that some of the bryophytes promoted growth of the shoot, some promoted growth of the root, while the others retarded the growth of both the roots and shoots. Thus, they observed both negative and positive influences of the bryophyte extracts on the germination of cress seeds. However, allelopathic plants have been reported to show more inhibitory effect on the root growth of other plants, this is because the root is the first organ to absorb allelochemical from the environment (Stachon & Zimdahl, 1980).

Conclusion

The aqueous extracts of *Archidium ohioense* and its litters used in this study enhanced seed germination in both *Zea mays* and *Vigna unguiculata,* with no inhibitory effect. Growing *Z. mays* and *V. unguiculata* in areas naturally inhabited by *A. ohioense* or its litter appears safe and beneficial. Nonetheless, further research is needed to explore the potential of *A. ohioense* and its extracts as eco-friendly, growth, and yield enhancers, offering a sustainable alternative to conventional synthetic bio-stimulants and phytohormones widely used to improve seed germination. The litters of *A. ohioense,* owing to its free and easy availability in the field especially during the wet or rainy season are therefore recommended to local farmers on their farms.

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