Fakulta zemědělská
a technologická
Faculty of Agriculture
and Technology

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE
FACULTY OF AGRICULTURE AND TECHNOLOGY

Department of Plant Production

Master Thesis

Study of Allelopathic Effects of Common Millet on Other Plants

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České Budějovice
2023

Declaration

I declare that I am the author of this graduation thesis and that I used only sources and literature displayed in the list of references in its preparation.

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Signature

Abstrakt

Proso seté (Panicum miliaceum) je významným zdrojem energie a bílkovin jak pro lidi tak i zvířata. V současnosti nejsou k dispozici prakticky žádné údaje o alelopatickém účinku prosa setého. Cílem práce proto bylo zhodnotit alelopatické účinky prosa na vybrané plodiny a navrhnout možnosti využití získaných poznatků. Laboratorní experimenty zahrnovaly hodnocení vlivu extraktů z rostlinných částí prosa a semen prosa na klíčivost semen a růst vybraných druhů rostlin, dále pak hodnocení autotoxicity extraktů prosa, hodnocení vlivu různé hustoty semen prosa na alelopatickou aktivitu a stabilitu alelochemikálií prosa. Nejúčinnějším extraktem inhibujícím růst vybraných rostlin byl extrakt z mladých rostlin prosa. Avšak alelochemikálie z proso setého se nezdají být vhodné pro potalčování plevelů kvůli jejich nízké stabilitě.

Klíčová slova: allelopatie, proso, semena, stabilita, autotoxicita, hustota, extrakt

Abstract

Proso millet (*Panicum miliaceum*) is important source of energy and protein for people and as well animals. At present, there are practically no data available on the allelopathic effect of proso millet. The aim of the work was to evaluate the allelopathic effects of common millet on selected crops and to suggest the possibility of using the acquired knowledge. Laboratory experiments included evaluation of the effect of extracts from plant parts of millet and millet seeds on seed germination and growth of selected plant species, as well as evaluation of autotoxicity of millet extracts, evaluation of the effect of different density of millet seeds on allelopathic activity and stability of millet allelochemicals. The most effective extract inhibiting the growth of selected plants was the extract from young millet plants. However, millet allelochemicals do not seem to be suitable for weed control due to their low stability.

Keywords: allelopathy, millet, seed, stability, auotoxicity, density, extract

Acknowledgment

First and foremost, I want to sincerely thank my teacher and thesis supervisor for her uncountable support and assistance which has made this project a reality. Her invaluable patience, thorough feedback, and guidance have been the brain behind the success of this work today. I could not have been able to complete this journey without her invaluable support.

I am also very grateful to my classmates and senior colleagues for their innumerable support which has impacted and inspired me to complete this project and made it a reality today.

Additionally, I want to thank my family members back home for their constant show of love, prayers, support, and assistance whenever needed. To be precise, I want to thank my late father who laid the foundation for my educational success today, and my mother, brothers, and sister.

Thank you to all of you who have inspired and impacted me positively. I deeply appreciate you all.

Content

Introduction

There is no single organism on Earth, its existence is always tied to interactions with other organisms in the ecosystem. These relationships are shaped by environmental influences on all organisms around us. We can find many different relationships in nature, some of which can be positive, neutral and others negative. However, in each case, each such relationship affects the surrounding organisms and is itself affected by them. Allelopathy - interspecific chemical coaction is also included among these relationships.

The plant kingdom includes about 300,000 species (Bold et al., 1980) that can release a mixture of compounds into the environment. Substances produced by plant secondary metabolism are essential for plant cooperation and aid in the natural protective process against natural enemies and rivals. Their existence can be impacted on the possibility of having these compounds available within their habitat. Allelopathy can affect the progression and turnover of species in agroecosystems, bacause agricultural production is severely limited by weed invasion.

Intensive farming practices in agriculture during the last 60 years caused a considerable population reduction in the number of species present in farming areas. Weed on its own is a big issue. Weed control in organic systems focuses on preventive methods of weed regulation and on the production of vigorous competitive crops. The aim of an organic farmer is not total elimination of all weeds but needs to keep the weeds at an economical threshold. Hence, allelopathy is a promising environment-friendly tool for weed management and it has great potential for use in organic rotation and organic weed control strategies. However, there is need for more understanding of this process for it to be applied successfully.

At present, there are practically no data available in the literature on the allelopathic effect of proso millet (*Panicum miliaceum*). Because millet is an important agricultural crop used also as a cover crop, as well as when sowing mixtures of crops and by intercropping, it is crucial to assess this plant both in seeds and individual parts in terms of its allelopathic effect.

1 Literary Review

1.1 Proso millet

1.1.1 Worldwide Significance of Millets

The millets are known as little cultivated cereals which has an annual lifecycle and are an important source of food, as well as feed and fuel (Kothari et al., 2005). Cultivated species of millets (Figure 1.1) include proso millet (*Panicum miliaceum*), pearl millet (*Pennisetum gluacum*), Kodo millet (*Paspalum scroblculatum*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria pumila*), little millet (*Panicum sumatrense*), and barnyard millet (*Echinochloa crus-galli*) (Bouis, 2000; Wen et al., 2014). In order, according to significance, millets are known as the 6th most useful cereals in the world, providing support to more than one-third of the earths inhabitants (Verma and Patel, 2012; Changmei and Dorothy, 2014). Millet is a very important crop in severe settings, such as those in India, Sub-Saharan Africa, and West Africa, where usual rainfall is typically less than 500 mm and the soil is sandy and slightly acidic (Changmei and Dorothy, 2014). The energy and protein supplied by millets are particularly important for people across various countries especially in China, Japan, and Africa (Rachie, 1975; Amadou et al., 2013).

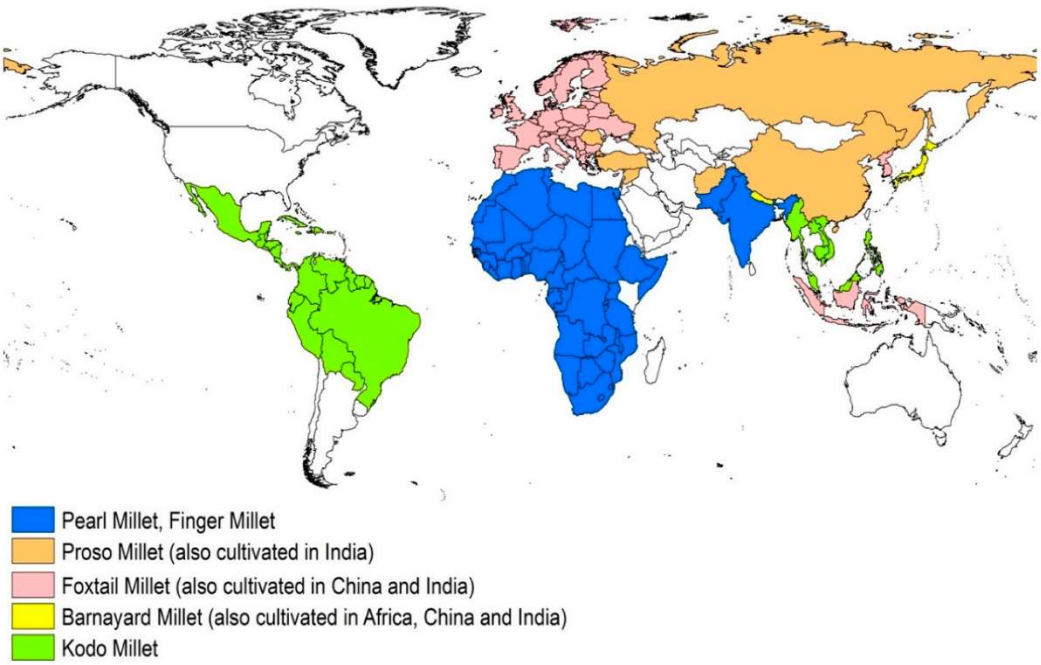


Figure 1.1: World geographical distribution of millet species growth (Saxena et a. 2018)

Millets are among the most reasonable harvests to support agriculture and food security in the least fertile and low-fertility environments. Millet crops are grown on peripheral land and under low input agricultural conditions (Amadou et al., 2013).

Proso millet is grown in northern China, Mongolia, Republic of Korea, southeastern Russia, Afghanistan, Pakistan, India, and southern Europe. The cultivation area of proso millet is 0.82 ha in Russia, 0.7 to 1.0 m ha in China (Wang et al., 2016), 0.5 m ha in India (Salini et al., 2010) and 0.20 m ha in USA (Habiyaramye et al., 2017). Among the millet species produced worldwide, proso millet is the most important species traded in the world market (Bhat et al., 2019).

1.1.2 Domestication and Spread of Proso Millet

Domestication of proso millet possibly began around 10000 BP (Figure 1.2). Todays archaeologists believes that proso millet original production started during the beginning of the Holocene, as global temperatures grew and hunter-gatherers faced new plants and conditions (Bettinger et al., 2007, 2010a,b).

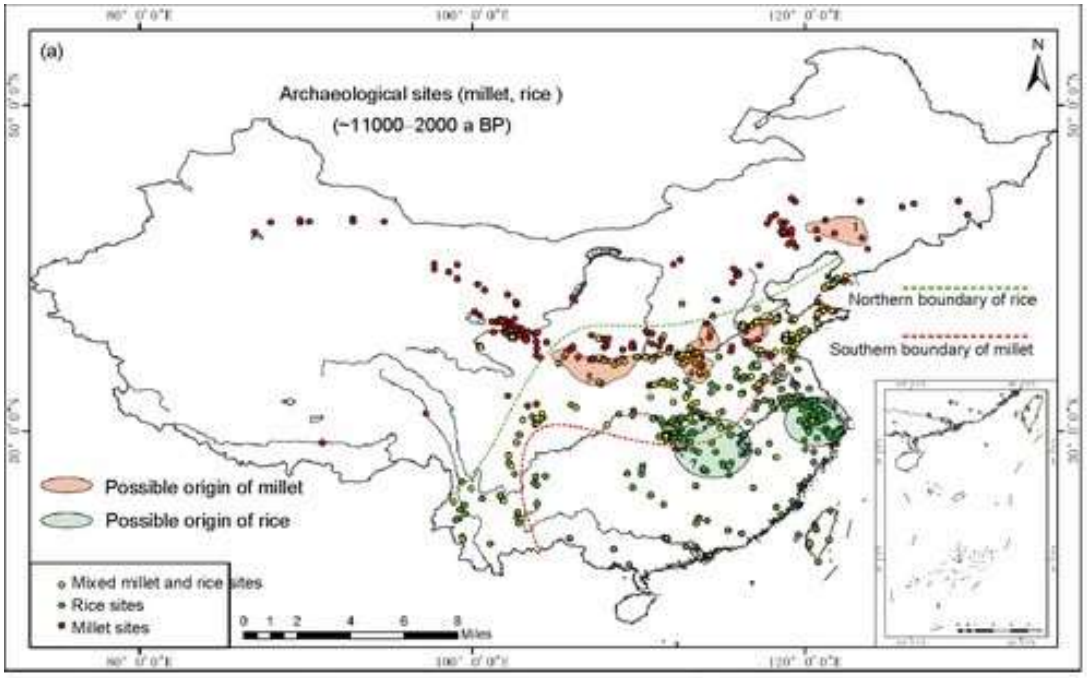


Figure 1.2: Representation map showing archeological areas of agriculture for china with respect to foxtail and common millet as well as rice and mixed crops (Lu, 2017)

Although archaeologists do not yet seem to have determined a specific date for the domestication of millet, they in most ways concur that it may have happened in China and Inner Mongolia (Bettinger et al., 2007., Zhao, 2005). Thanks to the fact that it is generally distributed in significant quantities in the Eastern parts of Asia, as well as regions high in altitudes (Marcott et al., 2013). Proso millet became a popular crop, especially in the northern lowlands of China. If the temperature rises in today's Himalayan region, it could allow the millet to grow again there. Proso millet spread to Kazakhstan and Pakistan during the fifth millennium of B. C. (Frachetti et al., 2010., Weber, 1991).

1.1.3 Taxonomy, Growth and Environmental Requirements of Millet

The wild ancestor of Proso has not yet been satisfactorily identified, but weedy forms are spread from the Aralo Caspian basin to Sinkiang and Mongolia. Originating seeds of proso millet into the centrally located countries as well as the eastern located countries in Europe can be traced as far back as the fifth millenium B.C. (Zohary and Hopf, 1988). The cultivated types have sometimes been classified into five races (Table 1.1) according to inflorescence morphology (Gomashe, 2017).

Table 1.1: Different races of proso millet sometimes called broomcorn millet (Gomashe, 2017)

Race	Inflorescence morphology	Characteristics
Miliaceum	Large, open inflorescences with sub-erect branches that are sparingly subdivided. Resembles wild *P. miliaceum* in inflorescence morphology.	These two races occur across the range of broomcorn millet cultivation, from Eastern Europe to Japan
Patentissimum	Its slender and diffused panicle branches are often difficult to distinguish from race *miliaceum*	
Contractum	Compact, drooping inflorescences	Highly evolved cultivars of broomcorn millet have more or less compact inflorescences
Compactum	Cylindrical-shaped inflorescences that are essentially erect	
Ovatum	Cultivars with compact and slightly curved inflorescences that are ovate in shape	

Proso millet (2n = 4x = 36) is predominantly a sel-pollinated grass of the end of spring and is the most developed as the last crop which is grown in summer (Baltensperger,

2002). Oval small seeds are called caryopsis and have size 3 x 2 mm. Stems are hollow with nodes (Figure 1.3). The external root structure is typical of Proso millet, plants are 30 cm to 100 cm high, with several tillers (Baltensperger, 2002).

Figure 1.3: *Panicum miliaceum* (Ibrahim and Peterson. 2014).

Proso millet is grown more north of other millets and is very well adapted to the conditions in the higher levels. The crop Proso Millet can as well be managed in unirrigated soils within dry areas with a normal rainfall of only 200–500 mm per annum (Ceccarelli and Grando, 1996).

From a temperature of 20° C reaching a level of 30° C, seeds of proso millet can germinate (Baltensperger et al., 1995; McDonald and Copeland, 1997; Amadou et al., 2013). With being a low-production C4 crop, it can effectively fix carbon even in the dry season, with temperature ranges that are high, even with very low concentrations of nitrogen or carbon dioxide situations (Baltensperger et al., 1995a). When the heat intensity exceeds 30 °C, the growth of the plant stem is interrupted, and blooming also stops (Herdrich, 2001; Sateesh, 2010; Changmei and Dorothy, 2014).

Soils that are sandy in nature can be effectively used for the growth of proso millet, as well as acid, salt, and soils that are low in fertility (Riley et al., 1989; Changmei and Dorothy, 2014). The growth of this crop is ineffective in water-flooded soils (Seghatoleslami et al., 2008). 5.5–6.5 pH levels are ideal for spreading proso millet (Lyon et al., 2008). Plants grown in soils with a pH of more than 7.8 show iron chlorosis.

The seeds attain maturity between 60-100 days old after it has been cultivated and sown (Baltensperger 2002). The colors of grains are different (white or white velvet, yellow or red, brown, grey). Varieties mostly present in the United States are the white type and trailed by the red-cultivated type (Hardman, 1990; Baltensperger, 2002; Williams et al., 2007; Changmei and Dorothy, 2014).

1.1.4 Nutritional and Health Benefits of Proso Millet

Grains of proso millet are rich in protein, and carbohydrates (Table 1.2) and can be classified in the groups of wheat, rice, and corn (Amadou et al., 2013; Saleh et al., 2013). Millet contains both macro and micro minerals (such as calcium, iron, potassium, magnesium, phosphorous, and zinc), which makes it a valuable crop (Devi et al., 2014; Gupta et al., 2014). Flours obtained from millet are not compatible with bread production but millet grains millet is gluten-free and is ideal for people who are gluten-intolerantive (Hulse et al., 1980; Thompson, 2009; Amadou et al., 2013; Santra, 2013). They contain a high measure of lecithin, which contributes to well-being by assisting

with reestablishing nerve cell work (Hulse et al., 1980; Pathak, 2013). Saleh et al. (2013) revealed that except for lysine and threonine, millet is a major source of basic amino acids, but in general, has high methionine content.

Table 1.2:| Proso millet (Panicum miliaceum L.), small millets, wheat, and rice (100 g) nutrient distribution (Habiyaremye et al., 2017)

Crop	Protein (g)	Carbohydate (g)	Fat (g)	Dietary fiber (g)	Mineral matter (g)	Calcium (g)	Phosphorus (mg)
Proso millet (*Panicum miliaceum* L.)	12.5*	70.4	3.1	14.2	1.9	14	206
Finger millet *Eleusine coracana* (L.) Gaertn.	7.3	72.0	1.3	18.8*	2.7	344*	283
Kodo millet *Paspalum scrobiculatum* L.	8.3	65.0	1.4	15.0	2.6	27	188
Foxtail millet *Setaria italica* (L.) P. Beauv.	12.3	60.9	4.3	14.0	3.3	31	290
Little millet *Panicum sumatrense* Roth ex Roem. and Schult.	7.7	67.0	4.7	12.2	1.5	17	220
Barnyard millet *Echinochloa esculenta* (A. Braun) H. Scholz	6.2	65.5	2.2	13.7	4.4*	11	280
Wheat *Triticum aestivum* L.	11.8	71.2	1.5	12.9	1.5	41	306
Rice *Oryza sativa* L.	6.8	78.2	0.5	5.2	0.6	45	160

Higher than major cereals and wheat, adapted from Saha et al. (2016).

The use of the crop has a variety of nutritional as well as clinical possibilities (Obilana and Manyasa, 2002; Yang et al., 2012). The consumption of proso millet is attributed to a reduction in the possibility of developing type 2 diabetes because the grains are high in magnesium content. Magnesium is a cofactor of several enzyme responses to control glucose and insulin discharge. Magnesium can also reduce the incidence of migraine headaches and heart attacks and is beneficial for people suffering from atherosclerosis (Shobana and Malleshi, 2007; Gélinas et al., 2008). The grain also contains a high quantity of phosphorus (Kumari and Sumathi, 2002). Phosphate promotes a healthy bone structure and is a very important part of ATP. A single cup of cooked millets provides about 24% of the daily requirement of phosphorus in the body (Liang et al., 2010; Devi et al., 2014).

Because millets are rich in fibers, and complex starch, they may play an important role in preventing cardiovascular diseases and malignant growth. Coulibaly et al. (2011) asserted it has a wide range of impacts applicable to medical benefits. Ordinarily, millet use can function to limit cholesterol problems in women during their post-menopausal period (Shahidi and Chandrasekara, 2013). Millet can also limit rapid skin deterioration which occurs as a result of old age in people (Pathak, 2013; Shahidi and Chandrasekara, 2013). What is more, it might prevent diseases that occur as a result of age (Pathak, 2013).

Millet can be cooked and prepared in a variety of ways and due to its mild taste, light color, and gluten-free quality, it is generating revenues for food companies in Europe and North America. It can be steamed to make a salad, or completely cooked like rice (Santra, 2013).

1.2 Allelopathy

Molisch used the word "allelopathy" in 1937 to show a direct or interconnected synthetic connection between plants that outcomes from the exchange of biochemical substances starting with one plant and then onto the next.

Relationships between one plant or microorganism as a result of biochemical interactions through the release of chemical compounds - secondary metabolites (allelochemicals), which influence plant growth and development either through a direct or indirect manner or through a negative or beneficial process was defined as allelopathy (Rice, 1984). Allelochemicals can be found in almost every plant and in many tissues, like leaves, stems, flowers, fruits, seeds, roots or pollen and may be released from plants into the environment by volatilisation, leaching, root exudation, and decomposition of plant residues (Chou, 1990).

During the 1970s, the investigation of allelopathy expanded essentially and has encountered colossal advancement since the mid of 1990s, turning into a significant point in agriculture, substance biology, plant science, soil science, climate, and different areas of investigation. Different allelopathic crops, significant in weed administration have been identified (Table 1.3).

Allelopathy is generally specific but is called autotoxication when it is intra-specific, e.g., when a plant produces allelopathic compounds that are harmful to the development of new plants of the same species.

Table 1.3: Examples of allelopathic crops

Crops	Allelochemical	Reference
Rice	Phenolic acid	Rimando et al., 2001
Wheat	Hydroxamic acids	Niemeyer, 1988
Cucumber	Benzoic and Cinnamic acids	Yu and Matsui, 1994
Black Mustard	Allyl isothiocyanate	Weston, 1996
Buck Wheat	Fatty acids	Weston, 1996
Sorghum	Sorgoleone	Netzley and Butler, 1986

Autotoxicity and allelopathy can perform critical jobs under both normal and controlled biological systems, due to their influence on the germination of seeds and the development of seeds (Rice 2012). Even though allelopathy known for many years, even hundreds of years, only a couple of allelochemicals have been identified

Allelochemicals can improve or obstruct the development of plants (Macias et al., 2003., Zeng et al., 2008). Allelochemicals are productive to control weeds in field crops (Iqbal and Cheema, 2007) because some allelochemicals have specificity (Bhadoria, 2011).

Weeds bring about losses in crops which is a genuine downside of contemporary agribusiness. Huge agricultural losses in the past can be attributed to incidences of weed and can be up to a tenth of total production. (Altieri and Liebman 1988). Even though allelopathy is typically considered to be a strain on farming, there is right now an effort that it be taken advantage of to help weed control in agroecosystems.

Ecological conditions can influence the allelopathic efects of a plant. The main natural factors that impact allelopathy incorporate UV radiation, temperature, water, nutrient accessibility, and rivalry stress (Croteau et al., 2000; Marchese and Figueira, 2005; Meiners et al., 2012). They can influence the bioavailability of allelochemicals in the

soil, water accessibility, and soil pH. For example, sorgoleone is strongly associated with soil colloids as an extraordinary lipophilic allelochemical with a log P coefficient of 6.1 (log octanol-water segment coefficient (Trezzi et al., 2006). The allelopathic L-DOPA (l-3,4-dihydroxyphenylalanine) and catechin are also unequivocally absorbed by soil colloids (Furubayashi et al., 2007).

Ecological factors can also alter allelochemicals availabilty and production in the soil. In non-sanitized soil, for example, DIBOA showed a half-existence of 43 h (Macías et al., 2005). Some flavonoid glycoside particles exudated by rice plants can be decomposed by soil microorganisms.

1.2.1 Allelopathic Compounds

It is difficult to identify every single compound recognized as allelochemicals (Rice, 1984).

The following 10 classes are used to categorize allelochemicals based on their structure and physical characteristics (Kelton et al. 2012):

1. Straight-chain alcohol, water-soluble organic acids, ketones, and aliphatic aldehydes,
2. Simple lactones,
3. Polyacetylenes and long-chain fatty acids,
4. Quinines (anthraquinones, benzoquinones, and complex quinines),
5. Cinnamic acid and its derivatives,
6. Phenolics
7. Coumarins,
8. Tannins,
9. Flavonoids,
10. Terpenoids and steroids.

The process of allelochemicals having a pathway into the environment happen with help of a variety of channels (Chon et al. 2006):

1. Washing of aerial parts i. e, leaves, flower and stem with rain, dew and fog,
2. Exudation of volatile compounds from aerial parts,
3. Exudation of roots
4. Decay of plant residues.

1.2.2 Types of Allelopathy

According to Seema et al. (2018), allelopathy types (Figure 1.4) are defined as follows:

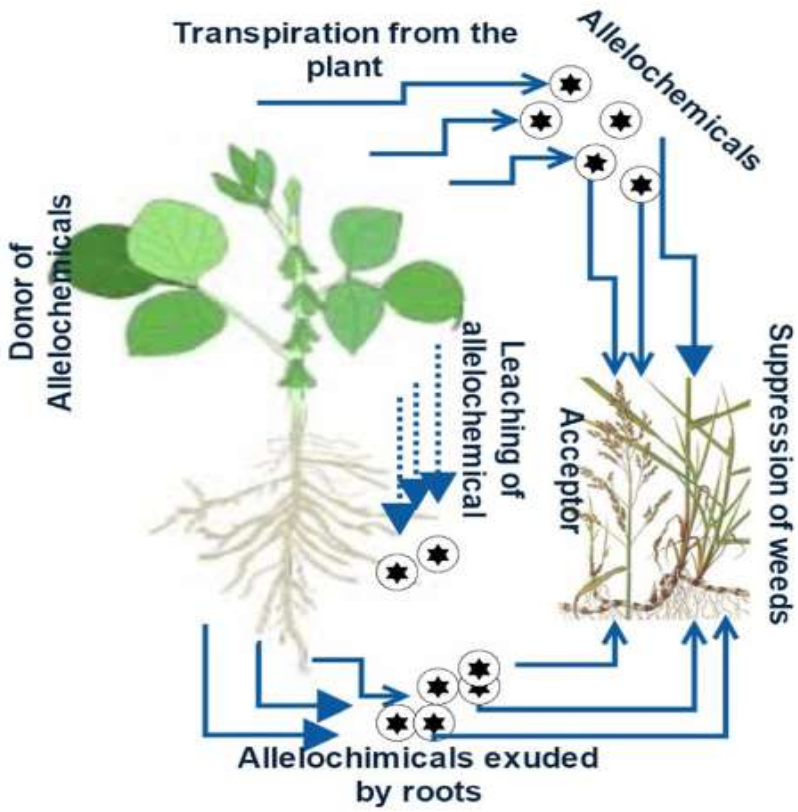


Figure 1.4: Allelochemicals as a process for weed suppression (Shah et al., 2016)

Autoallelopathy (autotoxicity): It is intra-explicit synthetic co-activity. Allelochemicals are harmful to the same species.

True allelopathy: It is production of substances into the climate that are harmful in the structure they are created by the plant.

Functional allelopathy: It is production of substances into the climate that are poisonous after adjustment by microorganisms.

Concurrent/direct allelopathy: It alludes to the immediate direct impact from the living plant to another nearby plant. It is likewise called the 'living plant effect'. For example (sorghum bicolor) smothers many weeds growing nearby.

Residual allelopathy: It is the impact on the plants growing in leaf litters, stem, and underlying foundations of the past plants.

1.2.3 Weed Allelopathy

In agro-biological systems, weeds contend with crop plants for nutrients, water, and place, meddle in crop care, diminish crop yield, and decrease their quality. Considering these qualities of weeds and their risks, it becomes basic to control them. Hand wedding requires huge work and time input. These days, chemical control gives a powerful procedure to weed control (Sodaeizadeh and Hosseini, 2012). Notwithstanding, the aimless utilization of herbicides to control weeds has brought about intense biological and ecological issues like herbicide obstruction, change in weed communities, contamination, and well-being perils because of poisonous buildups of herbicides in the soil, water, and food chain. Due to the disadvantaged effects associated with the use of herbicides, it is important to explore other ways to manage weeds (Nirmal Kumar et al, 2010).

In an agrosystem, allelochemicals are delivered by crop and also weed plant species. Many weed species revealed the allelopathic impact on crop species in different ways (Qasem and Foy, 2010).

Allelochemicals of the straw of ryegrass (*Lolium multiflorum* Lam.) caused a decrease of 34% in maize root development (Martin et al., 1990). The exudation of catechin from the spotted knapweed (*Centaurea stoebe*) impacts many crops (Bais and Kaushik, 2010). Powder and fluid concentrate from *Medicago sativa* bring negative effects on germination, nutrition take-up, and development of tomato (*Lycopersicon esculentum*) (Marian et al., 2017). The weak concentrations of *Taraxacum officinale* and *Cirsium vulgare* have significantly reduced the growth and development of corn and beans. *Taraxacum officinale* and *Cirsium vulgare* also suppress the germination of *Phaseolus vulgaris* (Marian et al., 2017). Some Solanaceae give the potential for weed biocontrol by producing and releasing allelochemicals from leaves, roots, bark, and seeds (Duke and Lydon 1987; Anaya-Lang 1989).

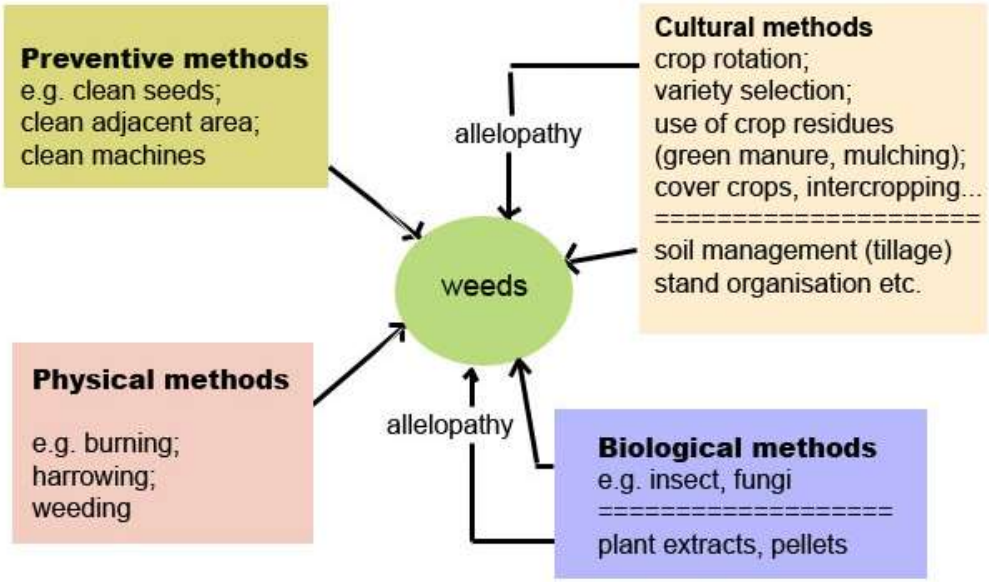


Figure 1.5: Utilization of allelopathy in organic weed management (Kalinova 2010)

Weed management uses preventive methods such as an appropriate crop rotation, precise soil bed preparation before sowing, narrow seed spacing, etc. Many of them include as well ways in which allelopathy (often together with competition) could play an important role (Figure 1.5). Crop allelopathic interactions may provide weed control by (a) use of allelopathic crops as cover crops, mulches, or green manure, (b) use of allelopathic plants in crop rotations, (c) crop mixtures and intercropping, (d) varieties with strong allelopathic potential (e) use of allelopathic crop water extracts and other agents.

Allelochemicals can help control other harmful factors E.g. the rice plant root let out of for example p-coumaric acid, assists with lessening fusarium problems (*Fusarium oxysporum* f. sp. *niveum*) in melon under mix with rice (Hao et al., 2010). Rapeseed (*Brassica napus* L.) diminished the amount of (*Xiphinema americanum*) nematodes in the field because of the exudation and hydrolyse of thyocyanates, isocyanates, and nitriles (Halbrendt 1996).

1.2.4 Allelopathy of *Poaceae*

Grasses of *Poaceae* are one of the very important family today (Heywood, 1978). As regards mans evolution, as well as the importance of grains to animals feed it has played an important role. Growing and managing grains has helped human advancement from 7000–10,000 years ago to evolve in advancement.

The fact that *Poaceae* is one of the most important and most widely "used" families in the world is one of the reasons for the extensive research on their allelopathic compounds. The kind and nature of their allelochemicals goes from phenolics to quinones, with a very important diversity in this range. The most clearly identified compounds can be divided into four groups: phenolic acids, hydroxamic acids, alkaloids, and quinones.

The most common phenolic acids are: p-hydroxybenzoic, syringic, vanillic, ferulic, p-coumaric, chlorogenic, caffeic, p-hydroxybenzaldehyde, gallic, protocatechuic, etc., in *Avena* spp. (Wardle et al., 1994), *Oryza sativa* (Alsaadawi et al., 1998), *Secale cereale* (Wójcik-Wojtkowiak et al.,1990), *Sorghum* spp. (Ben-Hammouda et al., 1995), *Triticum aestivum* (Wu et al., 2001, 2002), and *Zea mays* (Chou & Patrick, 1976).

Several authors identified the benzoxazinones (BOA, DIBOA, DIMBOA, AZOB, DIMBOA–glc, MBOA, Cl–MBOA, etc.) in different species, above all in*: Zea mays* (Friebe et al., 1997), *Aropyron repens* (Schulz et al. 1994), *Triticum aestivum* (Quader et al., 2001) and *Secale cereale* (Mwaja et al., 1995).

Some alkaloids have also been reported in *Hordeum vulgare* such as hordenine and gramine (Liu & Lovett, 1993). Sorgoleone is a very well-researched example of quinone due to its importance and its presence in the exudates in *Sorghum bicolor* (Weston & Czarnota,2001).

It is not clear by what procedure compounds of allelopathy of plants are released. Also, which pa a plant they can be identified is not fully specified. But in reality, we can find allelopathic substances in the grains, pollen, root exudates, decomposing straw, extracts from different plant parts, soil extracts, etc.

Probably the best-described allelopathy is in sorghum. However, information on allelopathy in proso millet is rare. Sorghum is a significant grain crop with the critical

potential to smother weeds (Glab et al., 2017). Harmful consequences of sorghum on crops have been known to farmers without knowing the real reason (Breazeale, 1924). Putnam and Duke (1974) have observed that sorghum is a deeply allelopathic crop due to its accumulation (in the field, the vegetation decreases to 95% except for weeds). Cheema (1998) observed that sorghum root buildups, joined with soil, stifled the development (dry weight) of weeds. In contrast, *Melilotus parviflora* development was promoted by sorghum deposits. Allelopathic sorghum inhibits the development of certain species, yet probably won't influence some species and may have stimulatory impacts on others (Cheema, 1998).

Sorghum showed a significant amount of allelochemical substances in the leaves, stems, and roots (Guenzi and McCalla, 1966). The composition structure of the sorghum deposits demonstrated the important content of phenolic acids, especially p-cumaric acid, along with ferulic, syringic, vanillic, and p-hydroxybenzoic acids. The sorghum was revealed to be harmful at harvest time, and decaying requires about 22 to 28 weeks (Guenzi et al., 1967). Sorgoleone, which is released from living sorghum, is phytotoxic for a few weeks (Netzly and Butler, 1986). Cheema (1988) found that sorghum harvested before blooming had no allopathic effects on wheat or weeds. The mature root, leaves, and stems of sorghum have had an extremely impressive allelopathic effect on weeds and wheat crops. According to Sene et al. (2001), absolute phenol potential of sorghum varies between 4 and 156 kg/ha in the soil of the plant and 1 to 16 kg/ha in the roots.

Gonzalez et al., (1997) demonstrated that sorgoleone was an astonishing inhibitor of electron transport in Photosystem II and that the allelochemicals of sorghum affect most cycles directly or indirectly which are related to development. Nevertheless, their effect was explicit about the species and subordinated to concentration.

2 Aim of the Thesis

The aim of the work was to evaluate the allelopathic effects of common millet on selected crops and to suggest the possibility of using the acquired knowledge.

3 Material and Methods

3.1 Material

In vitro bioassays were used to determine the influence of substances contained in different parts of the millet plant on the germination of other types of plants. Five repetitions of each treatment were used in each experiment.

The experiment was based on the utilization of the seeds of 8 different plant types:

Proso millet (*Panicum miliaceum* L.) Hanácká Mana and Unicum varieties,

Common oat (*Avena sativa* L.) Poseidon variety

Spring barley (*Hordeum vulgare* L), Bojos variety

Common buckwheat (*Fagopyrum esculenthum* Moench), Zita variety

Sown head salad (*Lactuca sativa* L.) , Safír variety,

Narrow leaf lupin (*Lupinus angustifolius* L.), odrůda Dalbor,

White mustard (*Sinapis alba* L.), Severka variety

Spring sown sweet peas *(Pisum sativum* L.), Oskar variety

Before experiments commencement, verification of individual species germination was done.

3.2 Field Experiment

The site of the field experiment was located on the experimental station of the Faculty of Agriculture of the University of South Bohemia in České Budějovice, (48° 58' 29" north latitude, 14° 28' 29" east longitude,380 m.a.s.l., sandy-loam soil, with a pH 6.4, and mean light intensity of 3750 MJ/m2). Proso millet (Unicum variety) was grown in three repeated cycles from 11 May in 2021. Seeds were sown in lines 12.5 cm wide, with the growth density being 250 plants/m2. Sampled whole plants (30) which were the materials for the laboratory experiment was done after attaining full maturity.

3.3 Laboratory Experiments

3.3.1 Effect of Extracts from Millet on Seed Germination and Growth of Selected plant Species ('extract-to-seed')

Collection of millet material and preparation of plant extract

Shoots of proso millet Hanácká Mana variety were obtained from the experiment which was laid out in the field just before it was to be harvested. A division of the collected plants was made with leaves separated into parts, the stems separated into parts, and the roots as well. Oven-drying of collected plant parts was done at 45°C to a constant weight. Then the samples were cut at approximately 5-10 mm pieces, and 10 g of plant samples were extracted with 100 ml of distilled water in a glass jar for 24 h at 20°C in the dark. The mixture was filtered through filter paper (Whatman, 0.25 mm). The filtrate was designated as full strength (100%).

Experiment

Young plant extract was prepared from whole millet plants grown in the spring of 2022 in two containers (hydroponics, 23°C). Harvesting of the plants was done at 1 month of age just at the stage of several true leaves, and drying at 45°C to a uniform weight, and then processed as field samples. Only water was used as the control.

Twenty-seeds of each tested species were planted in 9 cm sized Petri dishes that had been lined with Whatman No. 1 filter paper. Six milliliters of extract were added to individual Petri dishes and to limit evaporation, a covering of parafilm piece was placed on each Petri dishes. Petri dishes were managed in a growing chamber at a temprature measured at 25°C. After 5 days of incubation, seeds which germinated with radicles larger than 1 mm were noted, root and shoot lengths were also recorded, and the weight of fresh and dried (105°C 24 hours) seeds was determinated.

3.3.2 Autotoxicity Assessment of Millet Residue Extract

Millet variety Unicum was used as the receiver and Hanacka Mana as the donor. Each of the receivers' 25 non-surface-sterilized seeds were planted on a 9 cm petri dishes. Whatman No. 1 filter paper was used to line the petri dishes in one layer. Each donor variety extract was delivered to each petri dish in six millionths of a liter (or one-third

of its full strength), with distilled water (5 ml) serving as the control. The subsequent steps and evaluation followed the same routine as the earlier tests.

3.3.3 Effect of Proso Millet Seeds on Seed Germination of Selected Plant Species ('seed-to-seed')

Each dish then contained 20 millet seeds and 20 seeds of another species for small seeds, and 10 millet seeds and 10 seeds of another species for large seeds. As a control, a dish was filled with only the specified species of seeds. The filter paper was moistened by using distilled water in the amounts of 5 ml per dish. The subsequent steps and evaluation followed the same routine as the earlier tests.

3.3.4 Influence of Different Density of Millet Seeds on Germination and Growth Tested Species (seed after seed)

The different numbers of millet seeds Hanacka Mana variety (10, 17, and 25 seeds per dish) under identical circumstances as in the "seed-to-seed" approach, were put in a Petri dish. Removal of the seedlings of millet was done after five days and replaced by ten seeds of the tested species. Once more, the Petri dishes were placed into the growing chamber. The preceding experimental setup earlier utilised was adopted.

3.3.5 Stability of Millet Allelochemicals

The allelochemicals assumed to have been released by the roots of millets were examined also for their stability. As the donor variety, the proso millet Hanacka Mana variety was used. Millet seedlings (25 seeds per dish) were removed from Petri dishes after five days of germination and replaced with ten seeds of the tested variety (Unicum) after 0 days, 2 days, and 4 days. The subsequent steps and evaluation followed the same formulas as the earlier tests.

3.4 Statistical Analysis

The difference in allelopathic potential of proso millet was evaluated by analysis of variance with the post hoc Tukey HSD test in program Statistica 12.0. Statistically significant are values $P \leq 0.05$. The inhibition percentage was determined using the formula: (Control – Treatment) / Control x 100/1

4 Results

The result of the study of allelopathic effects of common millet on other plants is presented below. For this topic, five different experiments were carried out.

4.1 Effect of Extracts from Common Millet on Seed Germination and Growth of Selected Plant Species ('Extract-to-Seed')

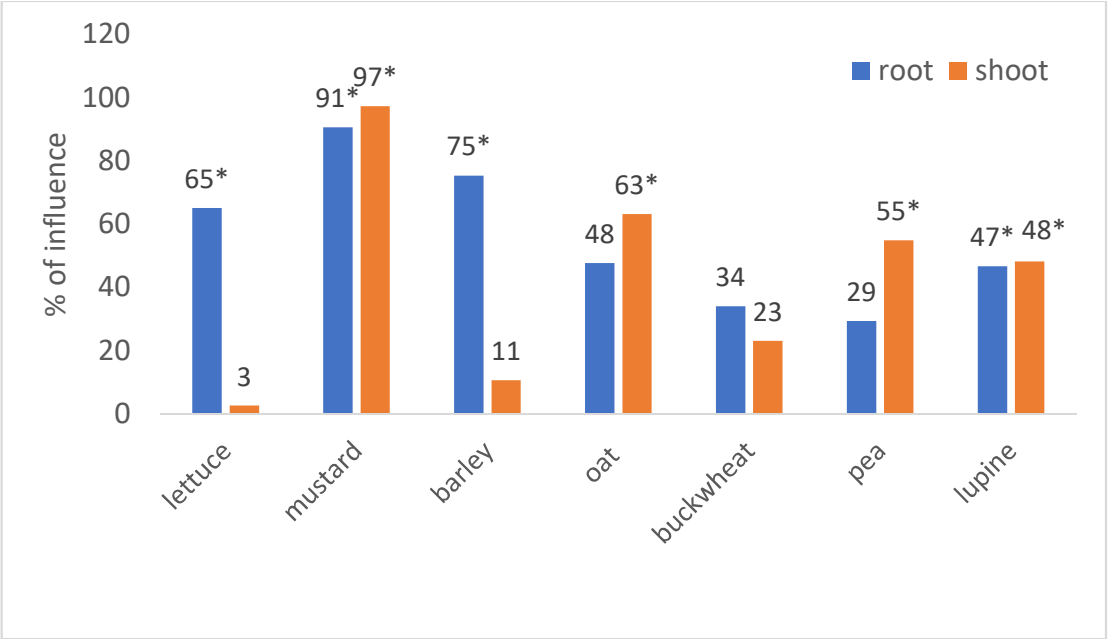
Young Plant Extracts

Table 4.1: The fresh weight, dry weight, germination percentage, length of root, and length of shoot of selected species in the control

Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Mustard	1.399 ± 0.072	0.144 ± 0.010	88.0 ± 9.08	3.304 ± 1.820	1.609 ± 0.974
Lettuce	0.358 ± 0.050	0.015 ± 0.001	76.0 ± 14.31	1.688 ± 0.997	1.141 ± 0.870
Buckwheat	2.686 ± 0.298	0.378 ± 0.037	37.0 ± 6.70	2.814 ± 1.771	1.486 ± 1.291
Pea	7.971 ± 0.679	2.211 ± 0.135	52.5 ± 21.92	1.811 ± 1.293	0.808 ± 0.573
Barley	1.608 ± 0.101	0.601 ± 0.033	52.5 ± 15.05	3.435 ± 1.390	1.638 ± 1.132
Oat	1.117 ± 0.611	0.376 ± 0.076	12.5 ± 6.25	0.743 ± 0.403	0.403 ± 0.112
Lupine	7.979 ± 0.852	2.124 ± 0.190	55.0 ± 15.56	3.995 ± 1.667	2.383 ± 1.284

Table 4.2: Influence of millet young plant extract on the fresh weight, dry weight, germination percentage, length of root, and length of shoot of selected species

Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Mustard	0.387 ± 0.025	0.156 ± 0.01	25.0 ± 17.32	0.32 ± 0.44	0.05 ± 0.03
Lettuce	0.301 ± 0.468	0.014 ± 0.01	5.0 ± 2.50	0.02 ± 0.05	0.03 ± 0.01
Buckwheat	0.889 ± 0.094	0.409 ± 0.05	45.0 ± 5.00	1.86 ± 1.25	1.14 ± 0.83
Pea	7.317 ± 0.853	2.803 ± 0.35	56.3 ± 16.54	2.56 ± 1.48	1.79 ± 1.22
Barley	1.702 ± 0.085	0.603 ± 0.03	50.0 ± 16.54	0.85 ± 0.57	1.47 ± 1.04
Oat	1.197 ± 0.115	0.601 ± 0.05	64.6 ± 25.26	0.38 ± 0.10	1.07 ± 0.94
Lupine	7.016 ± 0.717	2.077 ± 0.12	47.9 ± 3.61	2.13 ± 1.38	1.23 ± 1.02



Graph 4.1: Influence of young plant extracts of common millet on the root and shoots of selected species in % of inhibition (* $P \leq 0.05$)

The young plant extract of common millet showed great influence in inhibiting the growth and germination of the tested species (Table 4.1 and 4.2).

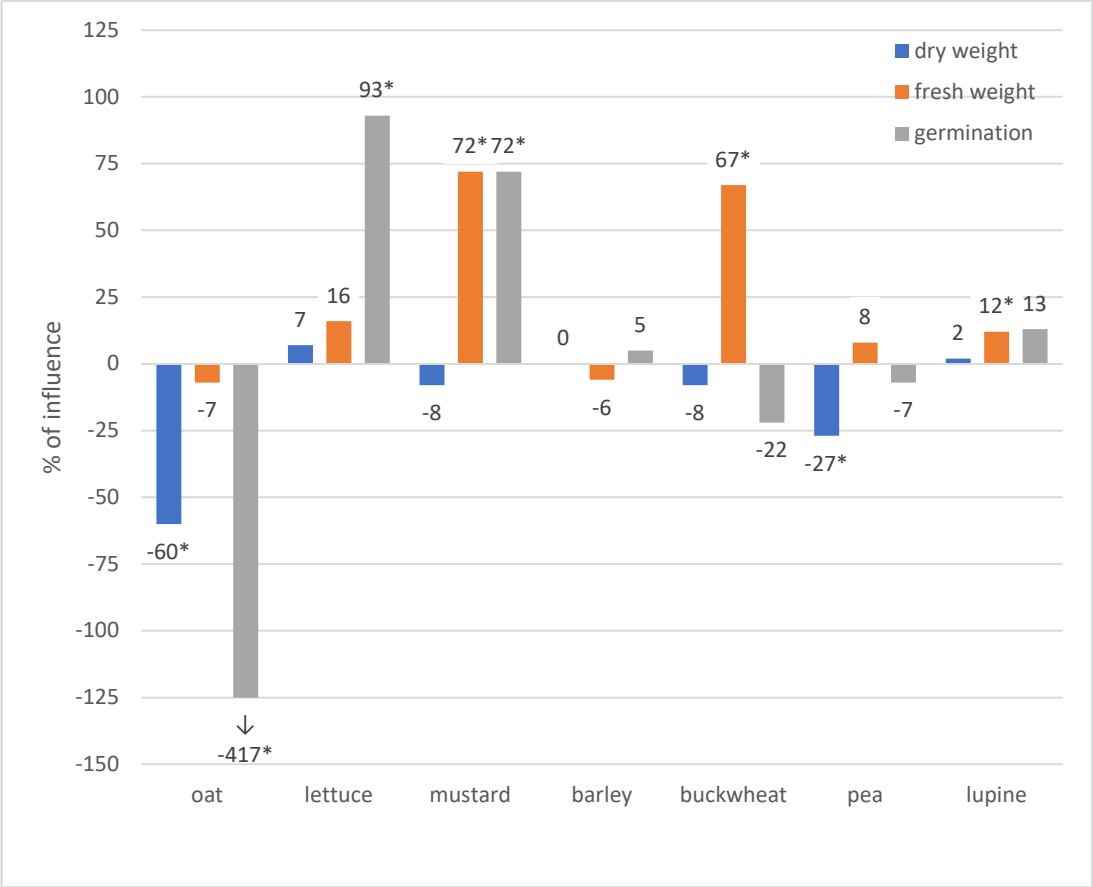
The obtained data showed that the young plant extract showed a significant influence on the root growth of lettuce, mustard, barley, and lupine (Graph 4.1). There was a great impact as a result of the treatment on the root growth of lettuce, mustard, barley and lupine. The highest root growth influence was obtained in mustard (97%) with the smallest value obtained in lupine (47%).

The treatment had a significant influence on the shoot growth of mustard, oat, pea, and lupine. On the shoot growth influence, the highest value was obtained in mustard (97%), and the smallest value was obtained in lupine (48%).

As regards the fresh weight and germination percentage, a significant influence was also observed on the tested species. The highest data on the fresh weight was observed on pea and the smallest value was obtained in lettuce. However, for the germination percentage, the highest value was determined in oats, and the smallest value was obtained in mustard.

There was no inhibitory effect on dry weight as noticed from the observed results but in oat, and pea, there was a stimulating effect. However, in the case of fresh weight,

there was a clear inhibitory effect in mustard, buckwheat, and lupine and no stimulating effect was observed. An inhibitory effect on germination was observed in lettuce and mustard, and a stimulating effect in oat (Graph 4.2).



Graph 4.2: Influence of young plant extracts of common millet on the weight and germination of selected species in % of inhibition (* $P \leq 0.05$)

Root Extracts

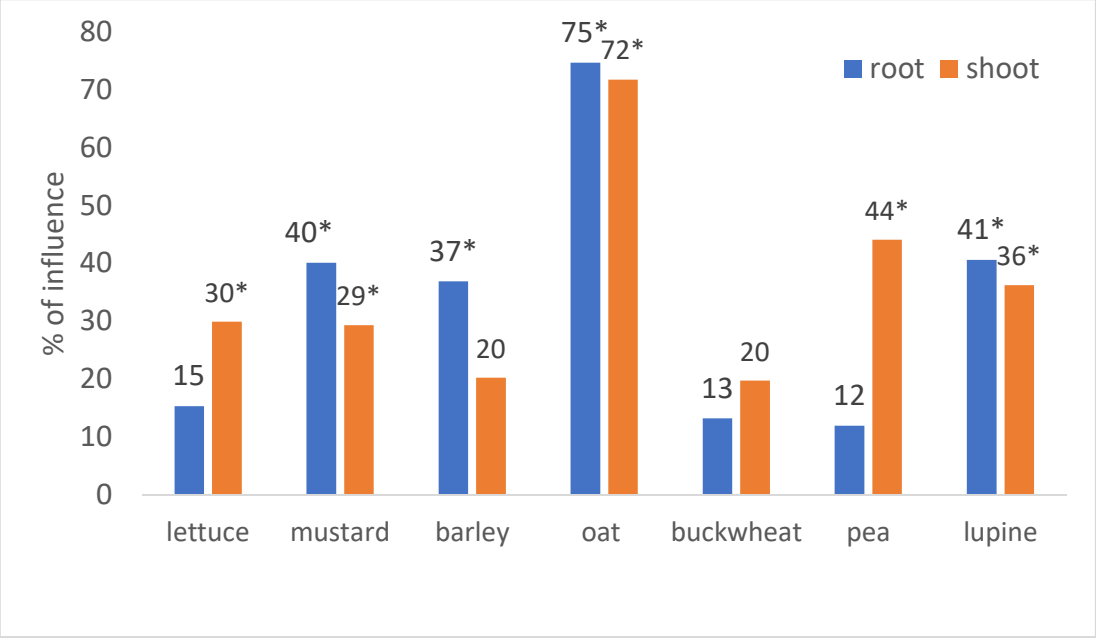
The results show that the root extract of common millet showed a significant influence in inhibiting the growth and germination of the tested species (Table 4.1 and 4.3).

The root extract of common millet showed a significant inhibitive influence on the root growth of the following species (mustard, barley, oat, and lupine) when compared with the control (Graph 4.3). The highest root growth influence was observed in oat (75%) and the smallest value in barley (37%). The effect of treatment with millet root extract was not significant in lettuce, buckwheat, and pea.

The treatment root extract of common millet had a significant inhibitive influence on the shoot growth of lettuce, mustard, oat, pea, and lupine. The influence of shoot growth was found to have the highest significant value in oat (72%), and the lowest significant value in mustard (29%). Only in barley and buckwheat was there no significant impact with the millet root extract treatment.

Table 4.3: Influence of millet plant root extract on the fresh weight, dry weight, germination percentage, length of root, and length of shoot of selected species

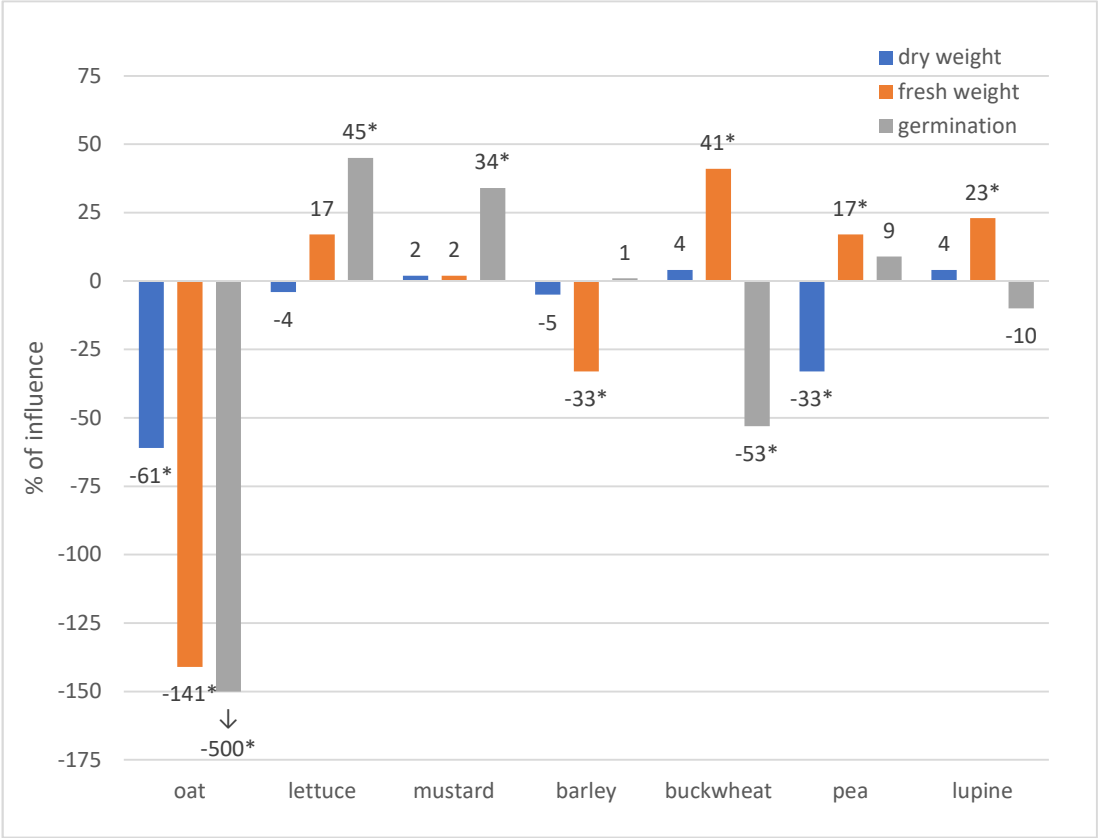
Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Mustard	1.376 ± 0.120	0.142 ± 0.016	58.33 ± 10.41	1.98 ± 1.57	1.14 ± 1.09
Lettuce	0.297 ± 0.043	0.016 ± 0.002	41.67 ± 10.41	1.43 ± 1.16	0.80 ± 0.83
Buckwheat	1.579 ± 0.201	0.364 ± 0.018	56.67 ± 7.64	2.44 ± 1.59	1.85 ± 1.12
Pea	6.625 ± 0.861	2.942 ± 0.496	47.92 ± 9.55	2.06 ± 1.62	1.44 ± 1.09
Barley	2.141 ± 0.136	0.633 ± 0.045	52.08 ± 3.61	2.17 ±1.24	1.31 ± 1.07
Oat	2.688 ± 0.193	0.608 ± 0.026	75.00 ± 6.25	2.94 ± 1.02	1.43 ± 1.04
Lupine	6.142 ± 0.387	2.041 ± 0.067	60.42 ± 13.01	2.37 ± 1.42	1.52 ± 1.16



Graph 4.3: Influence of root extracts of common millet on the root and shoots of selected species in % of inhibition (* P ≤ 0.05)

On the fresh weight and germination percentage, a significant influence of root extract of common millet was also observed on the tested species as compared to the control. Oats (72%) showed the highest stimulatory effect recorded.

There was no inhibitory effect on dry weight as noticed from the observed results but in oat, and pea, there was a stimulating effect. However, in the case of fresh weight, there was a clear inhibitory effect in lupine, pea and buckwheat, but in barley and oat, the effect was stimulating. An inhibitory effect on germination was observed in lettuce and mustard, and a stimulating effect in buckwheat and oat (Graph 4.4).

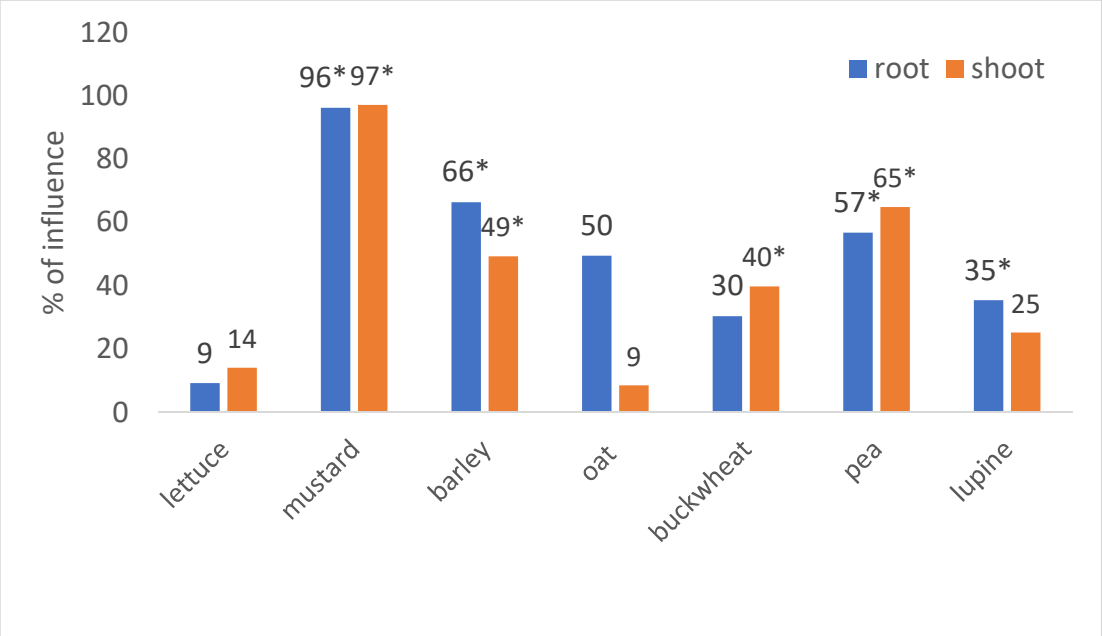


Graph 4.4: Influence of root extracts of common millet on the weight and germination of selected species in % of inhibition (* P ≤ 0.05)

Stem Extracts

Table 4.4: Influence of millet plant stem extract on the fresh weight, dry weight, germination percentage, length of root, and length of shoot of selected species

Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Mustard	0.515 ± 0.082	0.119 ± 0.013	7.00 ± 5.70	0.13 ± 0.06	0.05 ± 0.01
Lettuce	0.310 ± 0.123	0.016 ± 0.002	65.00 ± 16.20	1.53 ± 1.00	0.98 ± 0.73
Buckwheat	1.104 ± 0.207	0.314 ± 0.025	52.00 ± 22.53	4.04 ± 2.53	2.47 ± 2.20
Pea	7.651 ± 0.162	2.810 ± 0.211	83.75 ± 12.96	4.19 ± 2.05	2.30 ± 1.17
Barley	1.602 ± 0.136	0.588 ± 0.056	23.75 ± 11.18	1.15 ± 0.68	0.83 ± 0.50
Oat	1.301 ± 0.257	0.635 ± 0.024	66.25 ± 16.89	0.38 ± 0.22	0.44 ± 0.25
Lupine	6.542 ± 0.383	2.017 ± 0.123	43.75 ± 21.65	2.58 ± 1.77	1.78 ± 1.17



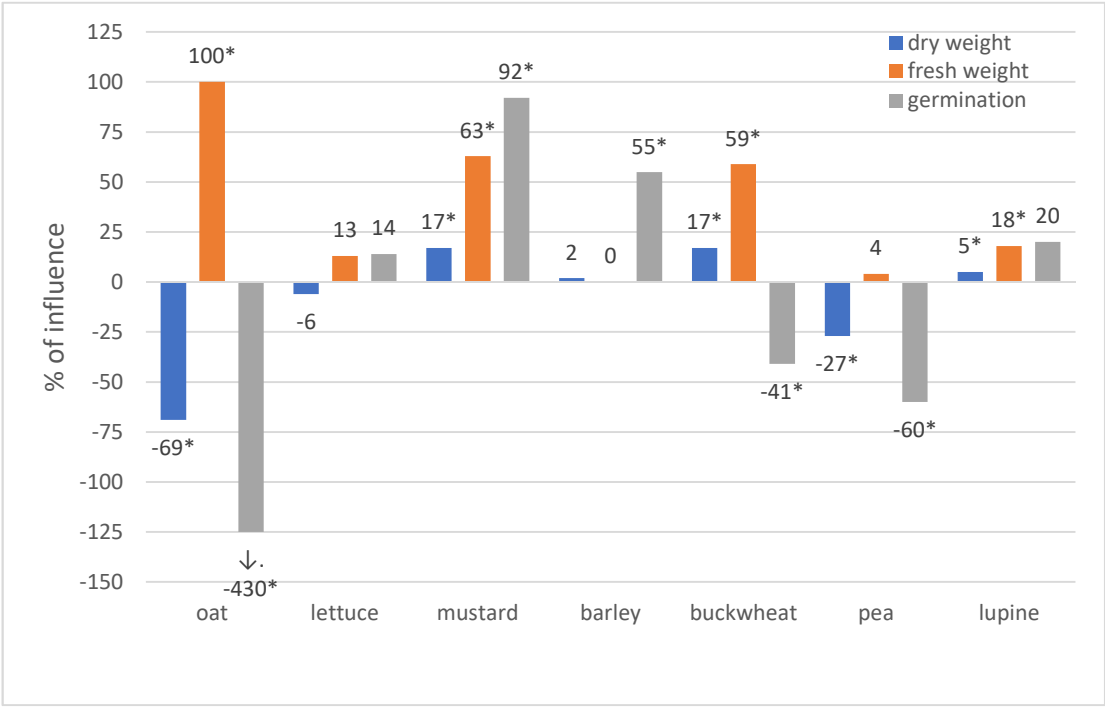
Graph 4.5: Influence of stem extracts of common millet on the root and shoots of selected species in % of inhibition ((* P ≤ 0.05)

The stem extract of common millet showed great influence in inhibiting the growth and germination of the tested species (Table 4.1 and 4.4).

The obtained data showed that the leaf extract showed a significant inhibition influence on the root growth of pea, barley, mustard, and lupine when compared with the control (Graph 4.5).

Furthermore, the data shows that the treatment had a significant influence on the shoot growth of mustard, barley, buckwheat, and pea. The highest significant root growth inhibition influence was obtained in mustard (96%) with the smallest value determined in lupine (35%). On the shoot growth influence, the highest value of inhibition was obtained in mustard (97%), and the smallest value was obtained in buckwheat (40%).

On the fresh weight and germination percentage of the tested species, a significant influence was also observed. The highest data on the fresh weight was observed on pea, and the smallest value was obtained in lettuce. However, for the germination percentage, the highest value was determined in pea, and the smallest value in mustard. There was an inhibitory effect on dry weight as noticed from the observed results but in mustard, buckwheat, and lupine a stimulating effect on oat, and pea. In the case of fresh weight, there was a clear inhibitory effect in oat, mustard, buckwheat, and lupine and no stimulating effect on the tested species. An inhibitory effect on germination was observed in mustard, and barley and a stimulating effect in buckwheat, oat, and pea (Graph 4.6).

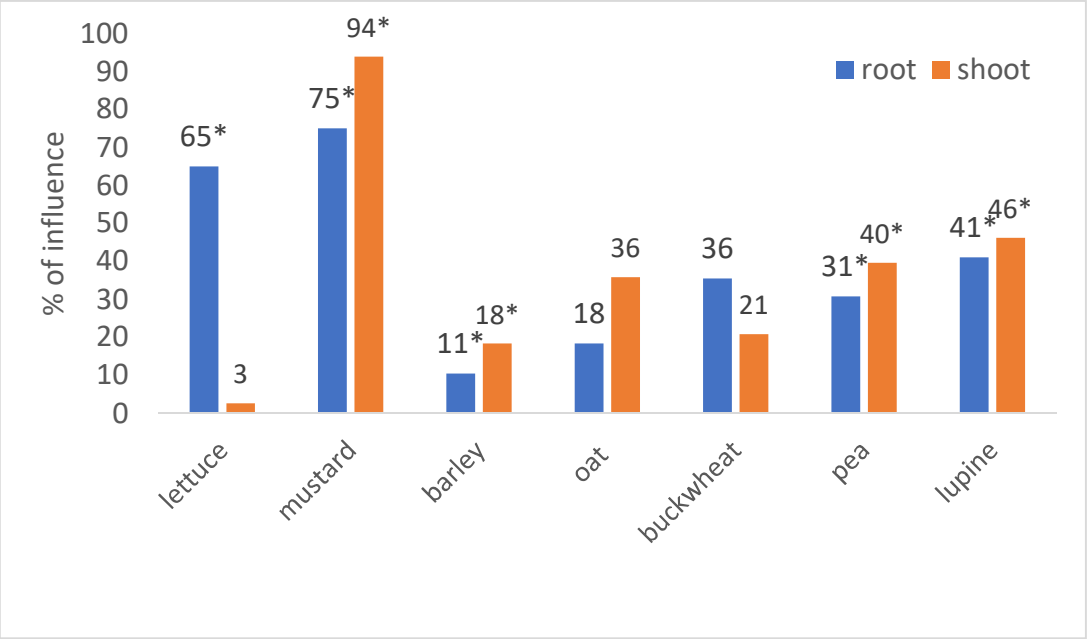


Graph 4.6: Influence of stem extracts of common millet on the weight and germination of selected species in % of inhibition (* $P \leq 0.05$)

Leaf Extracts

Table 4.5. Influence of millet plant leaf extract on the fresh weight, dry weight, germination percentage, length of root, and length of shoot of selected species

Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Mustard	0.466 ± 0.017	0.129 ± 0.012	27.5 ± 15.546	0.828 ± 0.750	0.099 ± 0.022
Lettuce	0.172 ± 0.027	0.017 ± 0.002	86.25 ± 17.017	0.591 ± 0.312	1.111 ± 0.673
Buckwheat	0.957 ± 0.211	0.339 ± 0.033	38.75 ± 14.361	1.814 ± 1.329	1.176 ± 1.070
Pea	6.828 ± 0.405	2.541 ± 0.267	62.5 ± 18.400	2.619 ± 1.867	1.336 ± 1.093
Barley	2.250 ± 0.204	0.592 ± 0.017	53.125 ± 11.968	3.075 ± 2.042	1.336 ± 1.068
Oat	1.370 ± 1.167	0.519 ± 0.034	7.813 ± 5.984	0.455 ± 0.334	0.194 ± 0.043
Lupine	6.779 ± 0.967	2.149 ± 0.127	53.125 ± 11.968	2.353 ± 1.403	1.283 ± 1.056



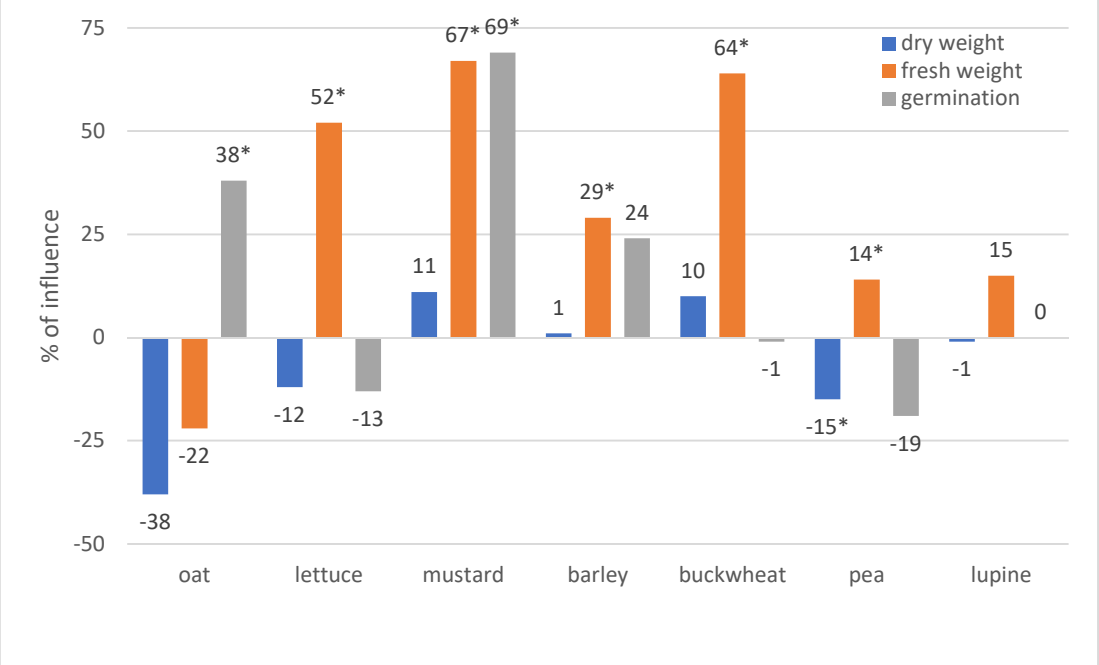
Graph 4.7: Influence of leaf extracts of common millet on the root and shoots of selected species in % of inhibition (*P ≤ 0.05)

The leaf extract of common millet showed great influence in inhibiting the growth and germination of the tested species (Table 4.1 and 4.5).

The leaf extract showed a significant inhibitive influence on the root growth of lettuce, mustard, barely, pea, and lupine (Graph 4.7).

Furthermore, the data shows that the treatment had a significant influence on the shoot growth of mustard, pea, barely, and lupine. On the shoot growth influence, the highest value was obtained in mustard (75%). The highest root growth influence was observed also in mustard (94%). The influence was not significantly different in oat and buckwheat.

On the fresh weight and germination percentage, a significant influence was also observed on the tested species as compared to the control. The highest data on the fresh weight was observed on pea and the smallest value was obtained in lettuce. However, for the germination percentage, the highest value was determined in lettuce, and the smallest in oat.



Graph 4.8: Influence of leaf extracts of common millet on the weight and germination of selected species in % of inhibition (* $P \leq 0.05$)

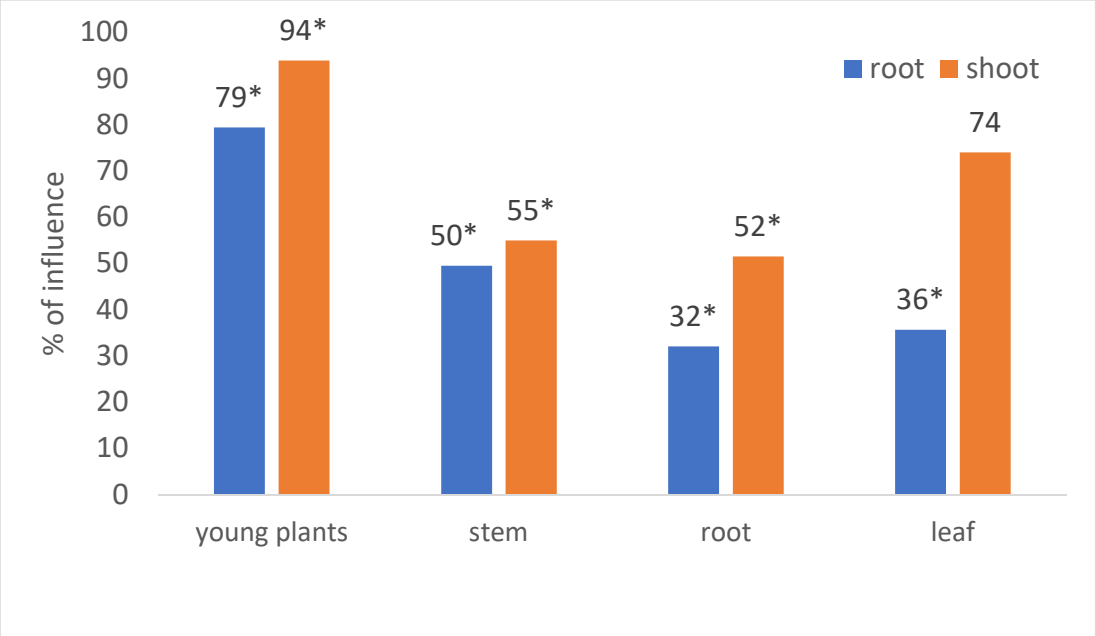
There was no inhibitory effect on dry weight as noticed from the observed results but in pea, there was a stimulating effect. However, in the case of fresh weight, there was a clear inhibitory effect in lettuce, mustard, barley, pea, and buckwheat. For the tested

species, the effect was not stimulating. An inhibitory effect on germination was observed in oat, and mustard but no significant stimulating effect was recorded on the tested species (Graph 4.8).

4.2 Autotoxicity Assessment of Millet Residue Extract

Table 4.6. Influence of millet plant extract on the fresh weight, dry weight, germination percentage, length of root, and length of shoot of millet

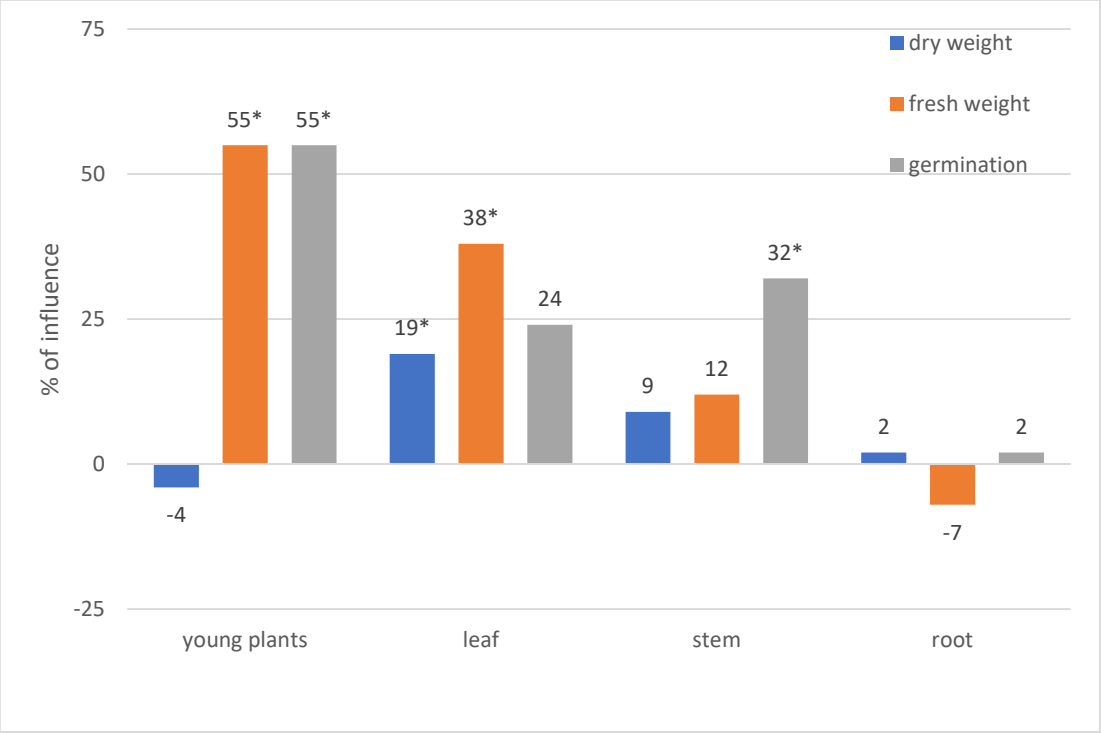
Extract form	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Millet leaf	0.255 ± 0.087	0.085 ± 0.003	50 ± 21.602	2.216 ± 2.251	0.834 ± 0.929
Millet stem	0.362 ± 0.095	0.094 ± 0.010	45.00 ± 8.66	1.74 ± 2.13	0.97 ± 1.34
Millet root	0.443 ± 0.072	0.102 ± 0.011	65.00 ± 5.00	2.34 ± 1.98	1.04 ± 0.97
Young plants	0.186 ± 0.005	0.108 ± 0.01	30.0 ± 8.66	0.71 ± 0.91	0.13 ± 0.24
Control -water	0.413 ± 0.053	0.104 ± 0.005	66.0 ± 11.93	3.447 ± 2.866	2.146 ± 1.955



Graph 4.9: Influence of common millet extracts from different plant parts on the root and shoots of common millet in % of inhibition (* P ≤ 0.05)

The various common millet residue extracts were used in this experiment (young plants extract, stem extract, root extract, and leaf extract) of autotoxic effects on the tested variety of common millet (Table 4.6).

Autotoxic effects of treatments ranked in order show that young plant extracts had the highest autotoxicity to common millet with a root impact of 79% and shoot impact of 94% (Graph 4.9). This was closely followed by the stem extract with 50% and 55% autotoxic effects respectively on the roots and shoots of common millet. The root extracts also showed great potential in exhibiting an autotoxic effect on common millet with observed data on the root at 32% and observed data on the shoot at 52%. For the leaf extract, autotoxicity was only established on the root impact of common millet at 36% while no significant impact was observed on the shoot.



Graph 4.10: Influence of common millet extracts of different plant parts on the weight of selected species in % of inhibition (* P ≤ 0.05)

Root extracts did not have an autotoxic effect on germination and weight, but the effect of the stem extracts was evident on germination but did not affect the weight. However, the inhibitory effect of the leaf extract was evident on the weight and on germination,

but it was at the limit of evidence, and the extract of young plants statistically significantly inhibited fresh weight and germination (Graph 4.10).

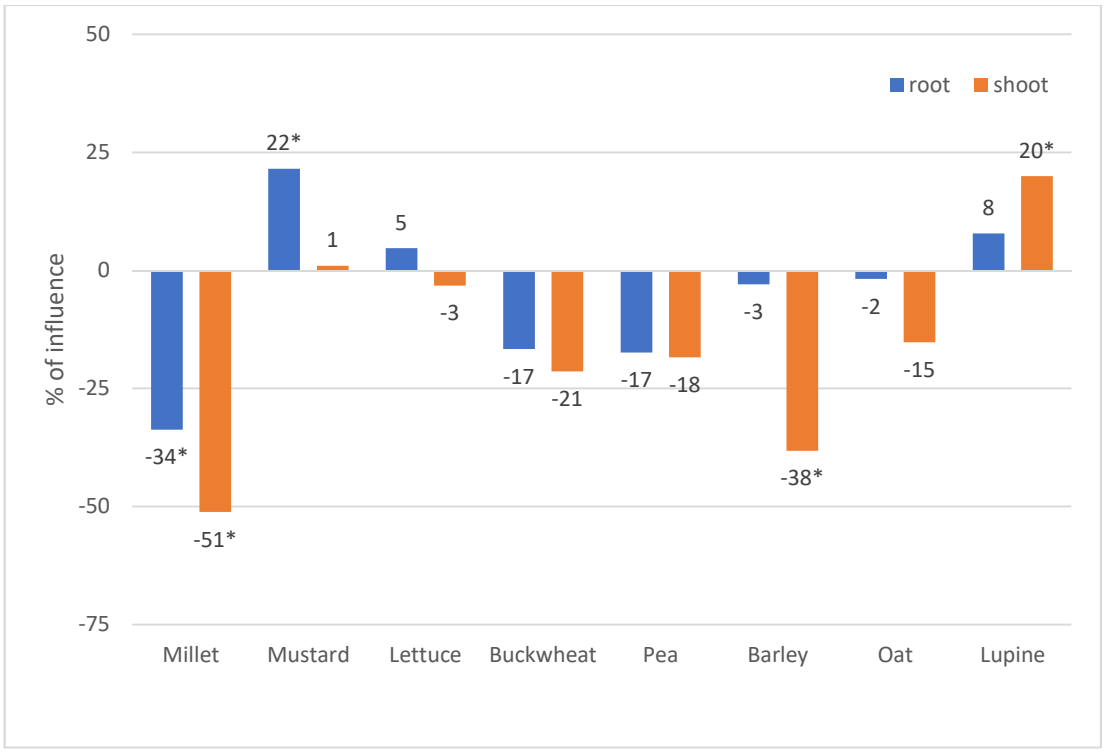
4.3 Effect of Proso Millet Seeds on Seed Germination of Selected Plant Species ('Seed-to-Seed')

Table 4.7. Influence of millet seeds on the fresh weight, dry weight, germination percentage, length of root, and length of shoot of selected species

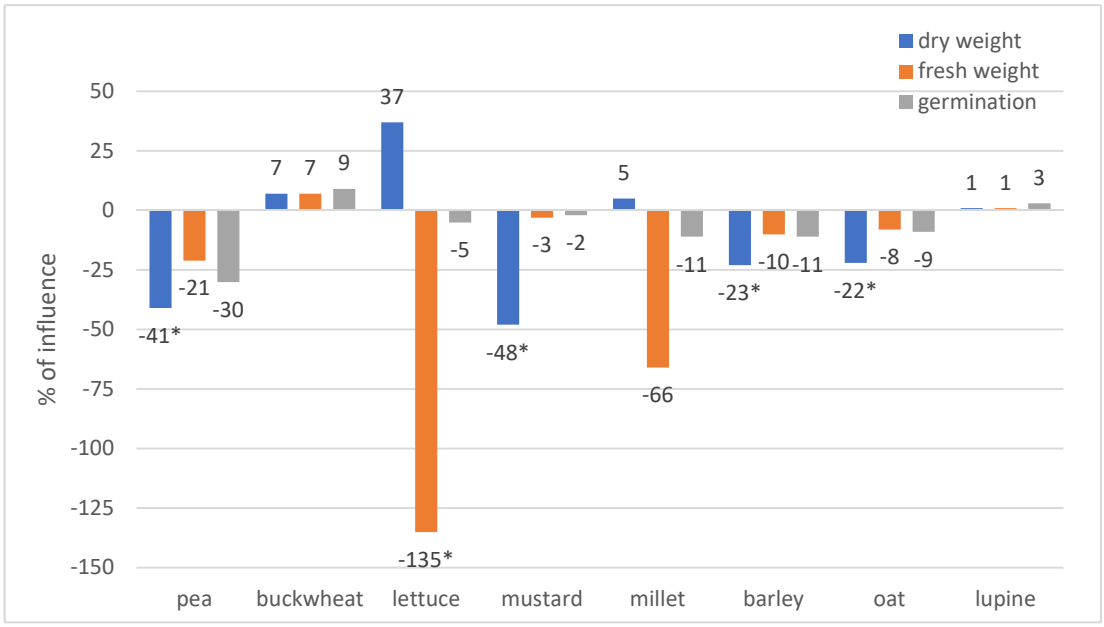
Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Millet	0.438 ± 0.248	0.098 ± 0.002	92 ± 4.472	3.246 ± 1.349	3.722 ± 1.874
Mustard	1.112 ± 0.086	0.128 ± 0.008	96 ± 8.944	3.514 ± 2.223	3.206 ± 1.451
Lettuce	0.483 ± 0.064	0.161 ± 0.233	94 ± 8.944	2.36 ± 0.865	3.016 ± 0.853
Buckwheat	1.187 ± 0.215	0.294 ± 0.038	52 ± 14.832	3.246 ± 1.753	2.51 ± 1.845
Pea	5.363 ± 0.755	1.808 ± 0.197	97.5 ± 5.590	5.478 ± 2.272	2.738 ± 1.160
Barley	2.152 ± 0.305	0.319 ± 0.021	97.5 ± 5.590	7.258 ± 1.776	5.29 ± 1.756
Oat	1.504 ± 0.225	0.299 ± 0.030	87.5 ± 12.5	5.75 ± 2.623	3.275 ± 1.811
Lupine	5.345 ± 0.674	0.957 ± 0.128	92.5 ± 11.180	2.695 ± 1.167	4.953 ± 1.982

Table 4.8: The fresh weight, dry weight, germination percentage, length of root, and length of shoot of selected species in the control

Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Millet	0.264 ± 0.040	0.104 ± 0.038	72.331 ± 29.047	2.428 ± 1.278	2.462 ± 1.220
Mustard	1.081 ± 0.152	0.086 ± 0.011	94 ± 8.944	4.48 ± 0.437	3.238 ± 0.496
Lettuce	0.206 ± 0.063	0.004 ± 0.004	89.231 ± 10.320	2.477 ± 1.128	2.923 ± 1.231
Buckwheat	1.276 ± 0.287	0.333 ± 0.025	56.923 ± 13.974	2.783 ± 1.555	2.068 ± 1.414
Pea	4.437 ± 1.144	1.287 ± 0.369	75 ± 33.072	4.667 ± 2.760	2.313 ± 1.159
Barley	1.960 ± 0.244	0.258 ± 0.031	87.5 ± 8.839	7.053 ± 2.575	3.828 ± 1.806
Oat	1.399 ± 0.194	0.245 ± 0.018	80 ± 11.180	5.648 ± 3.328	2.843 ± 1.745
Lupine	5.420 ± 0.561	0.948 ± 0.040	95 ± 11.180	2.925 ± 1.011	6.04 ± 1.626



Graph 4.11: Influence of common millet seeds on the leght of root and shoot of selected species in % of inhibition (* P ≤ 0.05)



Graph 4.12: Influence of common millet seeds on the weight and germination of selected species in % of inhibition (* P ≤ 0.05)

The observed data on the lenght of roots and shoots (Table 4.7 and 4.8) shows that there was observed mainly a stimulating effect in millet, pea, buckwheat and barely (Graph 4.11).

The observed data on the dry weight shows that there was no inhibitory effect on the tested seed species but only a stimulating effect was observed in pea, mustard, barley, and oat (Graph 4.12). For the fresh weight, there was also no inhibitory effect recorded across the tested seeds species but only a stimulating effect on lettuce. In terms of germination, there was no inhibitory or stimulatory effect recorded across the tested seed species.

4.4 Influence of Different Densities of Millet Seeds on Germination and Growth Tested Dpecies (Seed after Seed)

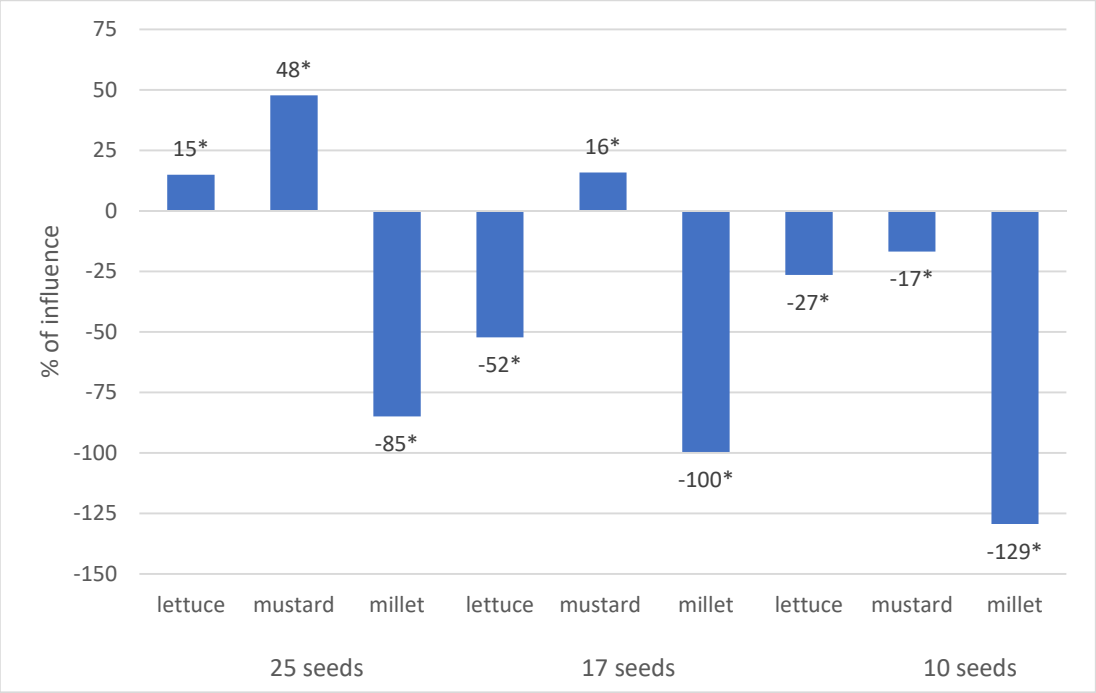
Table 4.9: Influence of different densities of millet seeds on germination and growth-tested species

Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
25 seeds					
Millet	0.247 ± 0.089	0.050 ± 0.004	68 ± 16.432	4.492 ± 3.339	2.664 ± 2.427
Mustard	0.752 ± 0.158	0.073 ± 0.005	98 ± 4.472	2.348 ± 2.212	4.456 ± 1.147
Lettuce	0.089 ± 0.039	0.008 ± 0.002	64 ± 30.496	2.108 ± 1.275	1.572 ± 1.012
17 seeds					
Millet	0.166 ± 0.055	0.0450 ± 0.003	64 ± 16.733	4.846 ± 3.203	2.588 ± 1.830
Mustard	0.855 ± 0.162	0.069 ± 0.002	98 ± 4.472	3.772 ± 2.426	3.772 ± 1.015
Lettuce	0.181 ± 0.083	0.008 ± 0.004	94 ± 8.944	3.772 ± 1.213	2.106 ± 0.876
10 seeds					
Millet	0.260 ± 0.052	0.046 ± 0.004	64 ± 15.166	5.57 ± 4.899	2.636 ± 2.294
Mustard	0.782 ± 0.193	0.070 ± 0.007	76 ± 18.166	5.234 ± 3.078	3.546 ± 1.667
Lettuce	0.137 ± 0.009	0.008 ± 0.002	76 ± 18.166	3.134 ± 1.710	1.876 ± 1.194

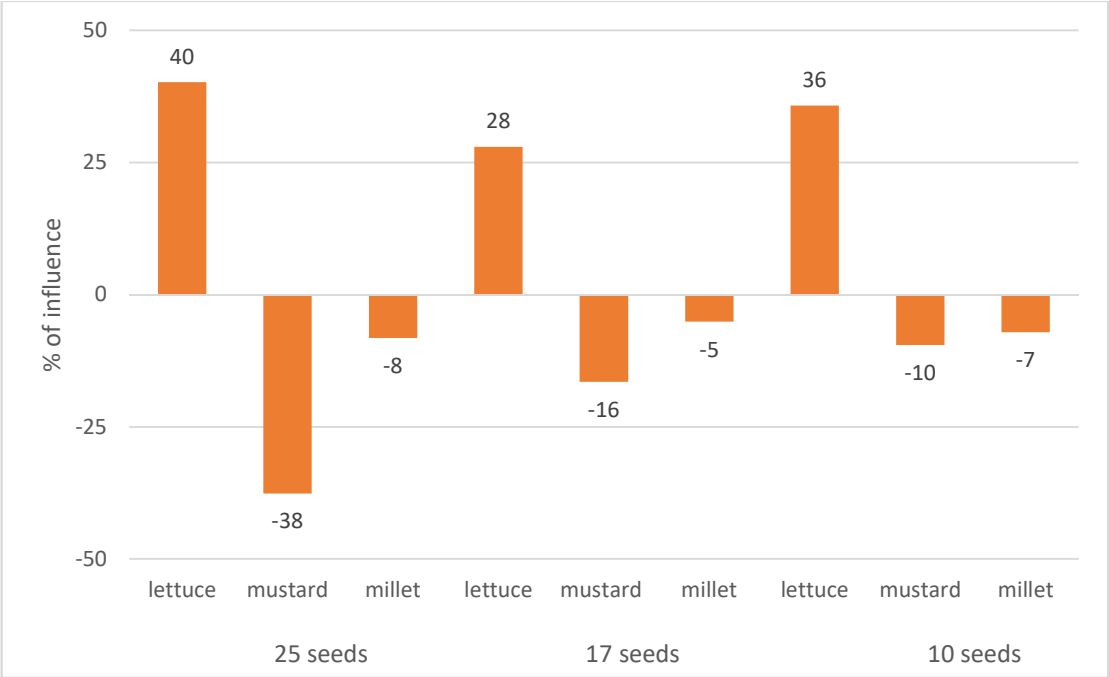
The test result showed that the different densities of millet seeds had a significant effect on the growth and germination of the tested seeds (Table 4.9). Root growth of the tested seeds showed significant differences across the various density levels. At 25

seeds density, an inhibition effect was observed in mustard, and lettuce, while a stimulating effect was observed in millet. At 17 seeds density, there was a stimulating effect observed in lettuce and millet while the only inhibition effect was observed in mustard. At 10 seeds density, there was no inhibition effect recorded but only a stimulating effect on mustard, lettuce, and millet (Graph 4.13). In the shoot growth, (Graph 4.14), dry weight (Graph 4.15), and fresh weight of the tested species (Graph 4.16), there was also no significant difference.

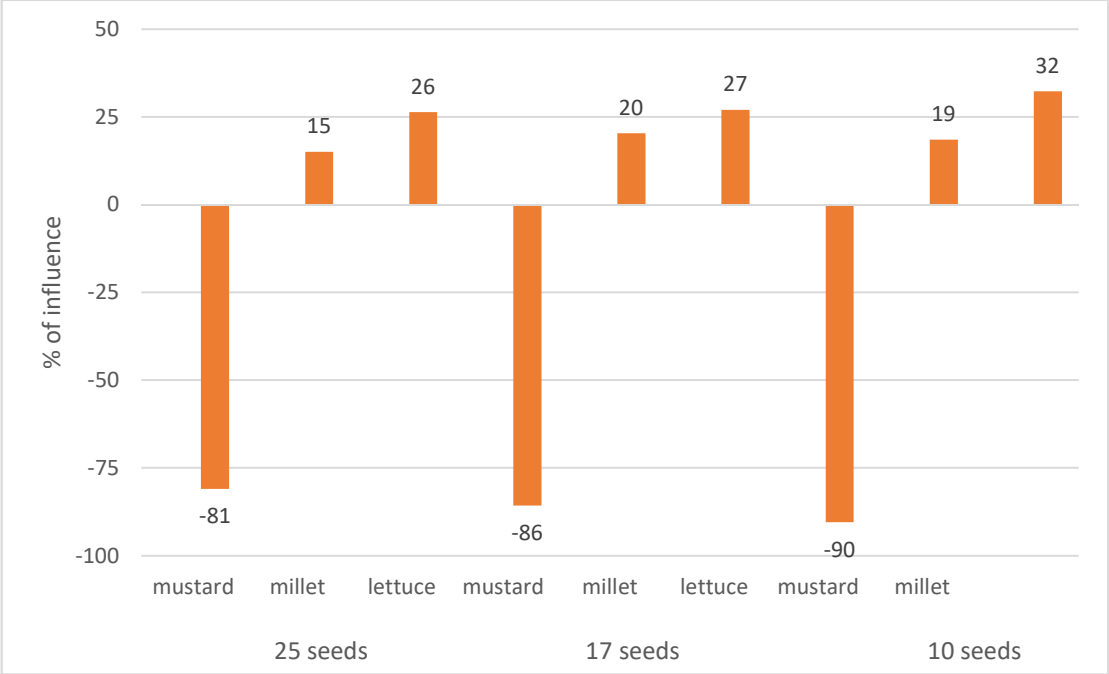
In terms of germination, no inhibitory effect was recorded at 25 seeds density but only a stimulating effect on mustard. At 17 seeds density, there was also no inhibitory effect recorded on the tested species but only a stimulating effect on mustard while at 10 seeds, there was an inhibitory effect recorded on mustard with no stimulating effect across the tested seed species (Graph 4.17).



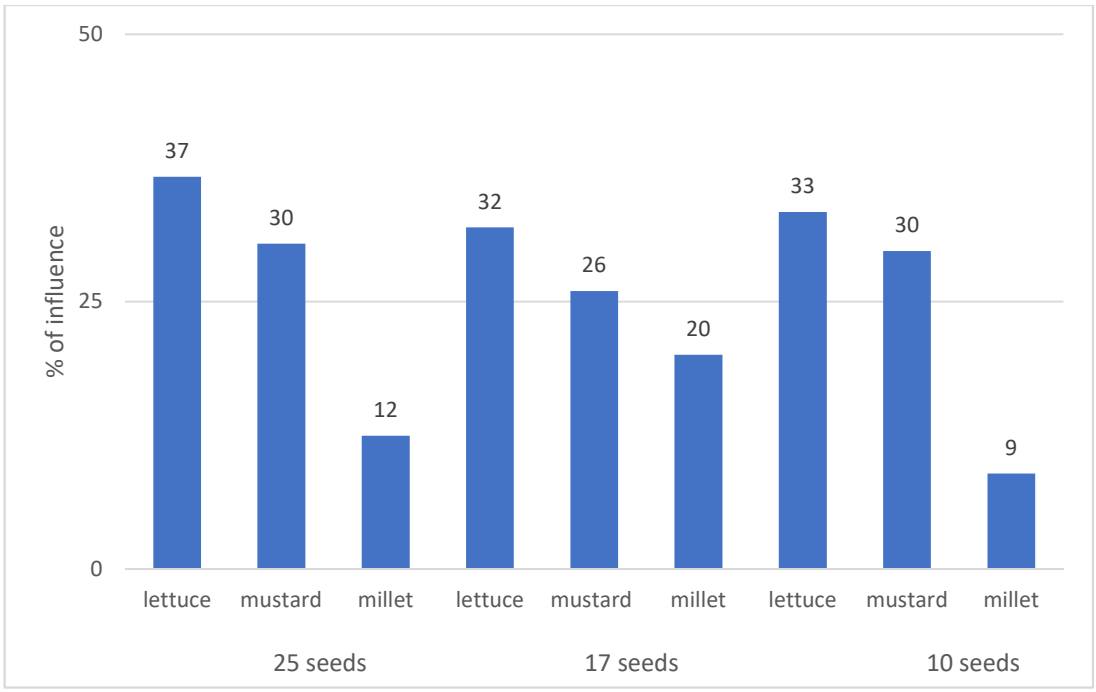
Graph 4.13: Influence of different densities (25, 17, 10 seeds) of common millet on the roots of selected species in % of inhibiton (* $P \leq 0.05$)



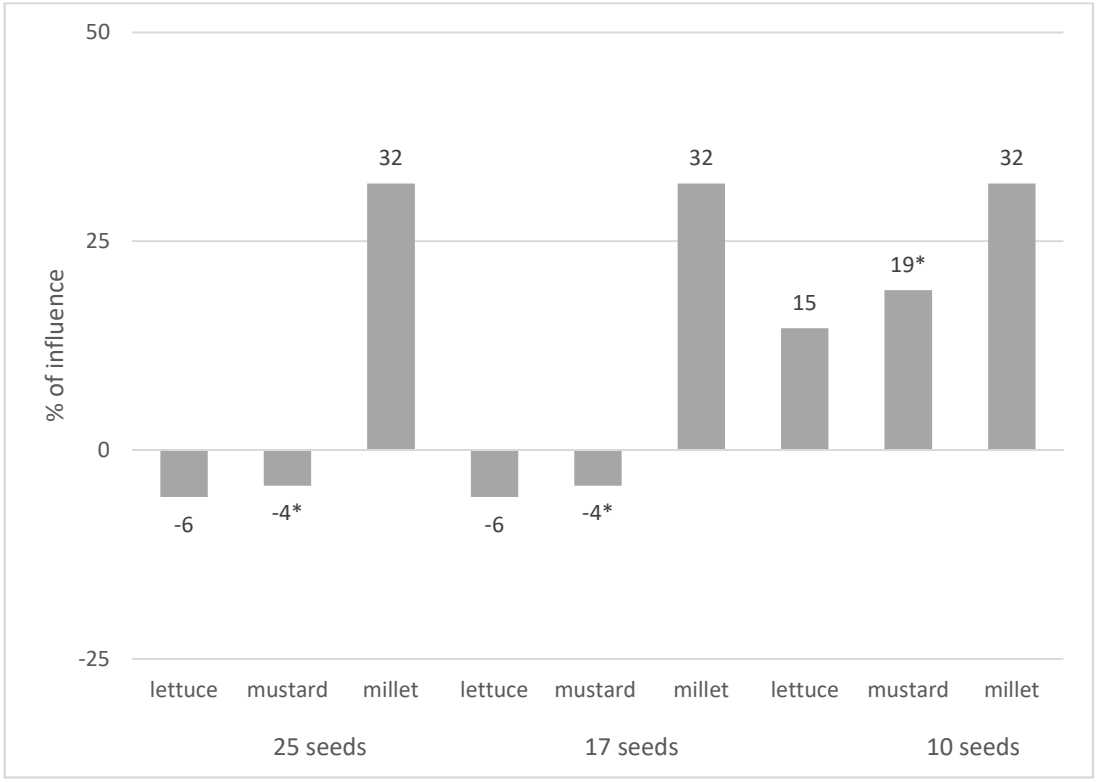
Graph 4.14: Influence of different densities (25.17, 10 seeds) of common millet on the shoots of selected species in % of inhibition (* $P \leq 0.05$)



Graph 4.15: Influence of different densities (25, 17 and 10) of common millet seeds on the dry weight of selected species in % of inhibition (* $P \leq 0.05$)



Graph 4.16: Influence of different densities (25, 17 and 10) of common millet seeds on the fresh weight of selected species in % of inhibition (* P ≤ 0.05)

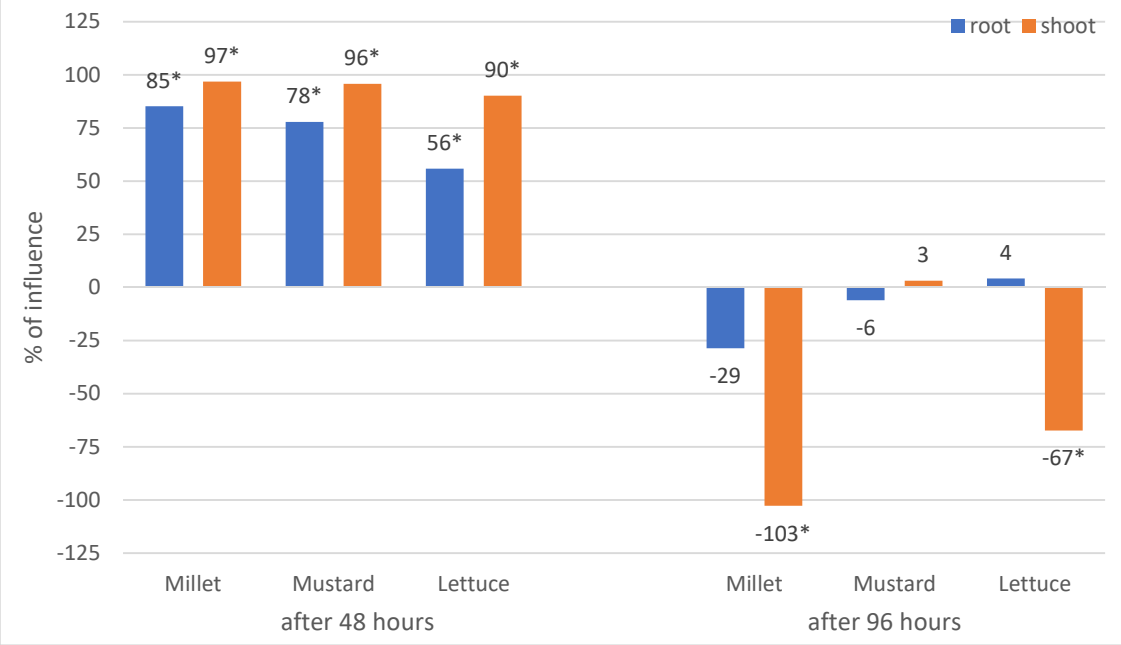


Graph 4.17: Influence of different densities (25, 17 and 10) of common millet seeds on the germination of selected species in % of inhibition (* P ≤ 0.05)

4.5 Stability of Millet Allelochemicals

Table 4.10: Influence of allelochemicals from millet seeds on weight, germination, and growth-tested species at different times after their production

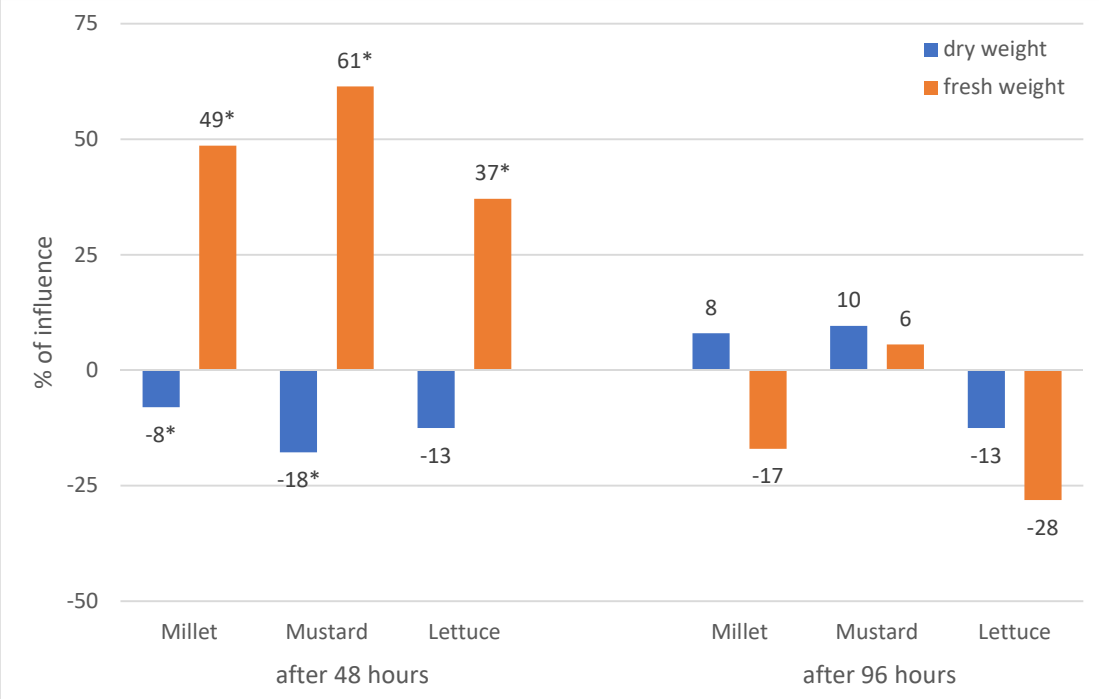
Species	Fresh Weight (g)	Dry Weight (g)	Germination (%)	Length of Root (cm)	Length of Shoot (cm)
Control – 0 hours after millet seed removal					
Millet	0.247 ± 0.089	0.050 ± 0.004	68 ± 16.432	4.492 ± 3.339	2.664 ± 2.427
Mustard	0.752 ± 0.158	0.073 ± 0.005	98 ± 4.472	8.908 ± 2.212	4.456 ± 1.147
Lettuce	0.089 ± 0.039	0.008 ± 0.002	64 ± 30.496	2.108 ± 1.275	1.572 ± 1.012
48 hours after millet seed removal					
Millet	0.127 ± 0.028	0.054 ± 0.004	26 ± 15.166	0.664 ± 0.685	0.084 ± 0.161
Mustard	0.290 ± 0.043	0.086 ± 0.008	74 ± 11.402	1.98 ± 1.370	0.19 ± 0.204
Lettuce	0.056 ± 0.009	0.009 ± 0.004	54 ± 23.022	0.932 ± 0.784	0.154 ± 0.178
96 hours after millet seed removal					
Millet	0.289 ± 0.042	0.041 ± 0.008	90 ± 14.142	5.78 ± 2.470	5.4 ± 5.475
Mustard	0.710 ± 0.104	0.066 ± 0.004	87.5 ± 5	9.448 ± 5.580	4.318 ± 2.110
Lettuce	0.114 ± 0	0.009 ± 0.000	60.0 ± 0.000	2.02 ± 1.846	2.63 ± 2.407



Graph 4.18: Influence of allelochemicals from millet seeds on the root, shoot leght of-tested species at different times after their production (48 and 96 hours) in % of inhibition (* P ≤ 0.05)

Allelochemicals from common millets seeds showed a low level of stability (Table 4.10).

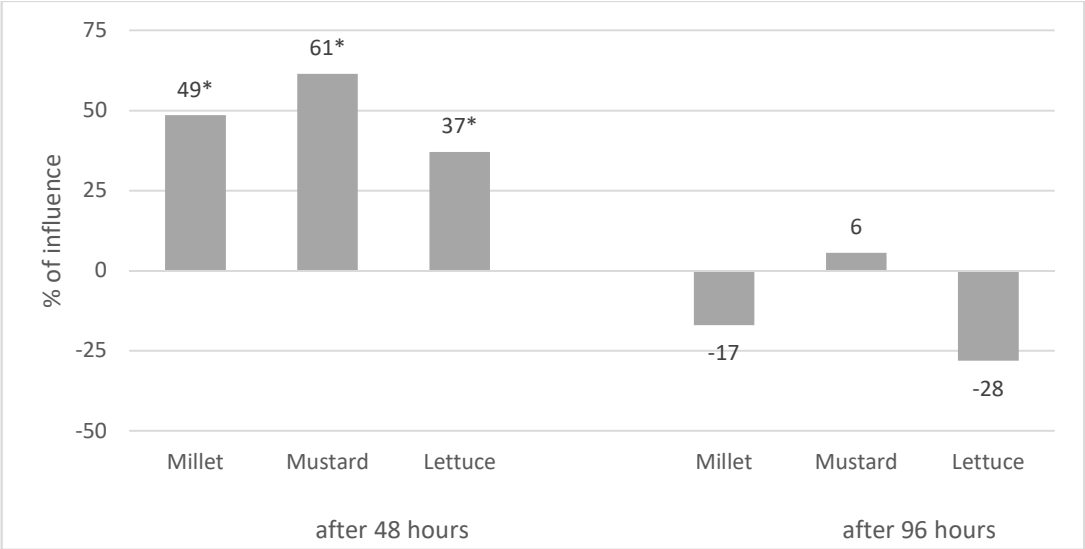
From the observed results, the inhibitory effect of millet seeds was demonstrated in all species after 48 hours on root and shoot (Graph 4.18). After 96 hours, the stimulating effect was demonstrated in the shoot of millet and lettuce.



Graph 4.19: Influence of allelochemicals from millet seeds on the weight of tested species at different times after their production (48 and 96 hours) in % of inhibition(* $P \leq 0.05$)

Millet seed's allelochemical impact on the weight of the tested species was clear after 48 hours for all the tested species in the case of fresh sprout biomass weight, but after 96 hours it was no longer evident (Graph 4.19). In the case of the dry weight of the sprout biomass, the effect was evident after 48 hours for mustard and millet, when the effect was manifested as stimulating. This indicates the low stability of allelochemicals from millet seeds.

The effect of allelochemicals of millet seeds on the germination of the tested species was not conclusive in lettuce, inhibition was recorded after 48 hours in mustard over millet, and after 96 hours the values found were no longer statistically significantly different from the control (Graph 4.20). This indicates little stability of allelochemicals produced by millet seedss.



Graph 4.20: Influence of allelochemicals from millet seeds on the germination of-tested species at different times after their production (48 and 96 hours) in % of inhibition (* $P \leq 0.05$)

5 Discussion

From the obtained results, it is apparent that the young plant extract and stem extract of proso millet had high shoot growth inhibition of the tested species. The highest effect was recorded with the young plant extract and closely followed by the stem extract.

In terms of the number of tested species inhibited on their shoot growth, the root extracts of proso millet ranked highest as they inhibited a greater number of species but were, however, less in effect when compared with the young plant extracts and stem extracts.

For root growth inhibition, the young plant extracts also had a higher effect on the tested species and were closely followed by stem extract. However, for the number of tested species inhibited, the leaf extract recorded the highest impact but had an overall lesser effect when compared with the young plant extracts and stem extracts inhibition percentage.

In terms of fresh weight inhibition, the highest effect was recorded with the stem extract and closely followed by the leaf extract. Also, for the dry weight, only the stem extract showed an inhibitive effect on the tested species.

For the germination percentage, the young plant extracts also had a higher effect on the tested species and were closely followed by stem extract. However, for the number of tested species inhibited, the stem extract recorded the highest impact and was closely followed by the root extract on the number of species but had an overall lesser effect when compared with the young plant extracts inhibition percentage.

Extracts of proso millet inhibited or stimulated the growth and germination of the tested species. This result was like earlier reports by Uludag et al., (2006), which observed that allelopathy covers effects which are stimulatory as well as inhibiting.

The allelochemicals present in the extracts of proso millet might have impacted root growth through cell division (Bhowmik and Doll, 1982). Phenolics presence can also be referred to as a reason for this. Aver and Goodwin (1956) previously explained the

idea that phenolics caused inhibition which was directly related to cell division. Elongation of the cells due to the extracts maybe were also affected as earlier reported by Tomaszewski and Thimann (1966) who in their conclusion reported that allelochemicals of different typed affected and reduced plant hormones and growth. This result agrees with previous reports of Kimbler (1973), and Gupta et al (1987) which reported the inhibitory effects of root and stem extracts of various crops.

Inhibition is most often seen in the roots. Thus, as a result of direct physical interaction of the roots of the plant and substrate, the root is more affected by a high phytotoxin concentration of the extract when compared to the shoot (Zucareli et al, 2019)

Extracts of proso millet in one way or another impacted the growth and germination of the tested seed species. Oliveira et al. (2015) examined allelopathy possibility as regards aqueous plant extracts of saccharine sorghum on germination and root growth of lettuce (*Lactuca sativa*). Similarly, common millet extract from young plants inhibited lettuce germination and the proso millet stem extract inhibited root growth of lettuce.

Costa et al. (2015) has stated previously that the grain obtained in terms of yield from soybean can increase when forage straw from sorghum is used. This agrees with the obtained results of this work, where the millet stem extract stimulated pea germination.

Also, there was a varying difference according to the different plant parts in relationship to the allelochemicals found in sorghum (Zucareli, et al., 2019) and this is same with results gotten in this study in which the different extracts from different parts of the proso millet exhibited an influence on the growth and germination of the tested seed species in one way or the other.

The young plant extracts of proso millet presented the highest effect in terms of inhibition as regards growth and germination on the tested seed species and this agrees with previous research when the aqueous extract of *S. bicolor* presented an allelopathic effect on germination and initial growth of kale seeds, with a higher inhibitory effect when extracts from pre-flowering plants were used, which corresponds to 60 days after emergence. Using straw from *S. bicolor* at this stage to aid in weed control is therefore advised (Zucareli, et al., 2019).

The highest autotoxicity effect in terms of the root and shoot growth, the fresh weight, and the germination percentage was recorded with the young plant extracts and closely followed by the stem extract. Extracts of proso millet inhibited common millet germination by 34–93%, radicle growth by 11–96%, and shoot growth by 18–97%. Similar studies revealed that the aqueous extract of wheat had a different level of autotoxicity and inhibited cole-optile growth by 5-20%, radicle growth by 15-30%, and wheat germination by 2-21% (Wu, et al., 2007).

On the effect of proso millet seeds on the root and growth germination of the tested plant species, it was apparent that the seeds of proso millet showed significant influence. The highest influence on root growth was recorded on mustard and closely followed by common millet.

The same species influence could be linked to the report of Singh et al. (1999) which outlined a mechanism and stated that "autotoxic allelochemicals aid to manage the population size of species with high density by increasing seed germination when seed density is raised. He also states that autotoxins can cause seed germination to slow down until the progenitor plant has a distance dispersed from the seed, thereby eliminating competition in parental and sibling forms.

For the shoot growth, the highest influence was recorded on millet and closely followed by barley. For the dry weight, only a stimulating influence was recorded with the highest effect recorded in mustard and closely followed by pea and for the fresh weight, a stimulating effect was recorded but only in lettuce. The effect of proso millet seeds on the tested species may be due to the presence of phenolic compounds and a variable amount of water-soluble inhibitory chemicals which either inhibit or stimulates growth. These phenolics appear to be among the key components as reported by Iannucci et al., (2012), and have been identified as allelopathic agents in the past by Mandava, (1984). It has been reported previously by many authors that phenolic compounds inhibit cell division (Aver, and Goodwin, 1956), reduce root growth (Bhowmik, and Inderjit 2003), slow the incorporation of amino acids (Baziramakenga et al., 1997), and also influence alterations in ultrastucture of cell walls (Li, et al., 2010).

It was apparent that millet seeds influenced the root growth of the tested species in dependence on the seed density. For the germination percentage, the highest influence was recorded at 25 seed density and 17 seed density, closely followed by 10 seed density.

Similar to this, Bouhaouel et al. (2015) discovered that using the approach of increasing barley seeds in terms of densities (0, 8, 19, and 25 seeds per Petri dish) decreased the growth of all responsive species (weeds and barley) in a dose-dependent way.

On the stability of millet allelochemicals, the inhibitory effect of millet allelochemicals decreased/changed to positive very quickly. After 96 hours, the effect as regards inhibition were no more noticed. In contrast with barley, allelochemicals process in terms of inbitory actions as regards growth of weeds in the radicle and coleoptile grew from 0 day till 6 days when barley seeds have been removed (Bouhaouel et al. 2015). This changes and growth in inbitory actions with time was bigger for coleoptile (38 % from 0 day to 6 days) when compared to radicle growth (29 %) (Bouhaouel et al., 2015). It was the same with millet, a greater effect was noted on the coleoptile growth.

Therefore, the poor stability of allelochemicals should be taken into account when allelochemicals should be used for weed inhibition. On the contrary, the low stability of allelochemicals brings possibilities for the inclusion of the cultivation of proso millet in the growing season. The obtained results agree with the statement that natural allelochemicals have poor stability and are easily affected by the growth status of plants. (Li, et al. 2011). The stability of allelochemicals may be affected by light, oxygen, and redox effect. So, oxidation, polymerization, and decomposition reaction of allelochemicals are all possible to occur (Wu, 2016).

Switchgrass (*Panicum virgatum* L.) aqueous extracts have been revealed to contain phenols, organic acids, neutral chemicals, and alkaloids, after accession in the past and these compounds have been bioassayed to examine their allelopathic potential. From all four chemical fractions, the highest effect in terms of allelopathy was produced by alkaloids (Shui et al. 2010). According to Wei et al., (2021), similar substances have been identified in proso millet which includes phenolic acids. Li e al (2021b) identified in proso millet grains 41 alkaloids.

6 Conclusions

- The proso millet extracts (young plant extracts, root extracts, stem extracts, and leaf extracts) were effective in inhibiting the growth and germination of some tested seed species.
- The most effective growth-inhibiting extract was the young plant extract, closely followed by the stem extract.
- The young plant extracts can be recommended as an ideal millet extract with a strong allelopathic potential.
- The young plant extracts had also the highest autotoxicity to common millet.
- The millet extracts had a stimulating effect on growth and germination in buckwheat, oat, and pea in terms of root and stem extracts.
- The seeds of proso millet did not significantly influence the germination of the tested species and had no inhibiting effect on both the dry weight and fresh weight of the tested seed species.
- The different density levels of proso millet seed significantly influenced only the root growth. The effect in terms of inhibition increased with the number of millet seeds from 10 to 25.
- Allelochemicals from proso millet showed an inhibition effect only at 48 hours. The stimulating effect resulted already after 96 hours.
- This shows that allelochemicals from proso millet may not be suitable for weed control due to their low stability.
- The results from this study conclude to show that the treatments by young millet plant extracts affected the growth and germination of seeds species tested and thus concluded to possess allelopathic abilities. However, more research should be done in the field to assess potential phytotoxic effects and physiological processes linked to allelopathic effects on the chosen seed species.

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