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# PHYTOCONSTITUENTS, PIGMENTS, GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS, AND ALLELOPATHY EFFECT OF *ALTERNANTHERA FICOIDEA* (L.) P. BEAUV

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**ABSTRACT**

**Objective:** The aim was to investigate phytoconstituents, composition of pigments, gas chromatography mass spectrometry (GCMS) and allelopathy of *Alternanthera ficoidea* (L.) P. Beauv.

**Methods:** Qualitative phytochemical analysis was carried out by the method of Paech and Tracey using five different solvent systems (D.W., ether, chloroform, ethanol, and methanol). Methanol leaf extract of this plant was analyzed using GCMS. Chlorophylls, carotenoid, and polyphenols were estimated by following standard methods. Allelopathy effect was studied using leaf leachets on seeds of jowar and mung.

**Result and Conclusion:** Investigation of above parameters in *A. ficoidea* showed presence of large amount of pharmaceutically important phytochemicals like tannins, saponins, phytols, carotenoids, xanthophylls and polyphenols. It indicates that this weed can be used in herbal medicines and dietary supplements. Since this weed is showing allelopathy effect it should be eradicated from fields before it spreads and occupies the place meant for crop plants.

**Keywords:** Allelopathy, *Alternanthera ficoidea*, Exotic weed, Phytochemicals.

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## INTRODUCTION

The plants are capable of activating defense mechanisms against other plants by producing hundreds of secondary metabolites. These substances have received special attention because of their agricultural potential as either natural herbicides or templates for new synthetic herbicides [1]. Polyphenols have a major role in defense mechanism and carotenoids protect chlorophyll from cellular damaging. A pigment is a molecule that absorbs and reflects light. The broad array of colors found in plant tissues such as leaves, flowers, and fruits, can be accumulated for by the presence of thousands of different kinds of plant pigments. Chlorophylls and carotenoids have been considered to be responsible for the color of green plants [2-4]. Chlorophylls are of interest to agriculture and ecology where they are indicators of the health status of individual plants and communities and are often used as a quantitative reference in physiological research. They are also permitted as food colors [5]. Chlorophylls have recently attracted interest as phototherapeutic drugs [6,7]. Many lines of research suggest that consuming diet rich in plant pigments may slow the process of cellular aging and reduce the risks of some types of diseases such as cancer, heart diseases, and stroke [8]. Identification of natural plant components could contribute to the discovery of allelopathic agents. Allelopathy is a sophisticated mechanism of plant defense.

Amaranthaceae comprises 65 genera and approximately 1000 described species that originate from the tropical, subtropical and temperate zones of Africa, South America, and Southeast Asia. The genus *Alternanthera* is a prominent member of this family and includes 80 species. *Alternanthera ficoidea* (L.) P.Beauv (synonym *Alternanthera tenella* Colla) [9] is an invasive alien weed found everywhere in India.

## METHODS

Specimens were collected from Satara and adjoining areas identified with standard literature and expert taxonomist [10].

### Preparation of samples for phytochemical analysis

Leaf, stem, and roots were shade dried for 3 weeks and then pulverized using an electric blender. The powdered material was stocked in an airtight glass container. A total of 1 g of dried leaf material was extracted in 100 ml each of cold distilled water, ether, chloroform. Ethanol and methanol for 48 hrs at 37°C. This was then filtered with Whatman No. 1 filter paper. The filtrate was concentrated on a steam bath to obtain extracts used for qualitative phytochemical analysis.

### Preliminary screening

The phytochemical screening of the plant extract was carried out by following the method of Paech and Tracey [11]. MeOH extract was used for gas chromatography mass spectrometry (GCMS) analysis.

### Pigment and polyphenol estimation

Chlorophylls [12], carotenoid [13], and polyphenols [14] were estimated by following standard methods.

### Preparation of leaf leachete for study of allelopathy

Leaf leachete was prepared by soaking 100 g of dry leaf powder in 500 ml of distilled water for 24 hrs. Then, filter through Whatman's No. 1 filter paper and allowed to evaporation. The same powder reextracted twice in fresh water and evaporated filtrate is pooled to 100 ml with D.W. and stored in refrigerator in amber colored bottle for further use, leaf leachetes of 1%, 2%, 5%, 10%, 15%, and 25% concentrations were prepared by further dilutions of stock solution.

### Seed germination bioassay

The healthy seeds of mung bean and jowar were used for seed germination bioassay. Seeds were surface sterilized with 0.02% aqueous mercuric chloride for 2 minutes, thoroughly washed with distilled water and 20 seeds were kept in each sterilized petriplate (9 cm diameter) lined with filter paper. The filter papers in petriplates were moistened with 10 ml of respective concentrations (1%, 2%, 5%, 10%, 15%, and

25%) of leaf leachet. The seeds placed in petri plates moistened with 10 ml distilled water were considered as control. The experiment was arranged in triplicate by using seed germination chamber at 26°C and natural light and dark period. A number of germinated seeds were recorded every day. The germination percentage, root and shoot length, vigor index, fresh, and dry weight of seedlings were recorded on the 7$^{th}$ day. Mean time for germination is an index of seed germination speed and velocity, and mean daily germination (MDG) an index of daily germination speed and calculated by germination speed formula [15] was calculated for a week.

**RESULTS AND DISCUSSION**

Plants are rich in a wide variety of secondary metabolites tannins, saponins, quinone, anthraquinone, steroids, glycosides, terpenoids, triterpenoids, flavonoids, alkaloids, coumarins, phenolic compounds, and essential oils with antimicrobial properties [16,17]. Plant synthesizes a wide range of chemical compounds which are classified based on their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites.

Secondary metabolites are synthesized during secondary metabolism of plants. Phenolic compounds are the most widely distributed secondary metabolites, ubiquitous in the plant kingdom. The great majority of active phenolic compounds isolated from higher plants are flavonoids and phenolic acids. Studies on phenolic compounds have shown a wide range of biological activities such as anti-inflammatory, hepatoprotective, antioxidant, antitrombotic, vasodilating, and anticarcinogenic [18,19]. All secondary metabolites have a specific function as like saponins have antifungal activity [20].

The previous preliminary phytochemical analysis showed presence of all type of phytochemicals while tannins and saponins were detected in moderate amount [21]. Preliminary phytochemical analysis of *A. ficoidea* shows the presence of alkaloids, flavonoids, cardiac glycosides, terpenoids, tannins, anthraquinones, and saponins (Table 1).

GCMS analysis confirmed the results as shown in Table 2. It showed the presence of five major groups. 3,7,11,15-tetramethyl-2-hexadecen-1-ol6, 9,12,15-docosatetraenoic acid, 9,12,15-octadecatrienoic acid, methyl ester and phytol are the groups which are pharmaceutically important. Phytol is one among the five compounds of the present study. 9,12,15-octadecatrienoic acid, (Z,Z,Z)-linolenic acid (R/T 20.06) possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n-Hexadecanoic acid - palmitic acid (R/T 17.25) can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Phytol-diterpene (R/T 19.67) is an antimicrobial, anticancer, anti-inflammatory and diuretic agent [22] as in *Cassia italic* [23]. Rani *et al.* [24] observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al.* [25] in *Mimosa pudica* leaves. A similar result was also observed in the leaves of *L. camara* [26]. It was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result, there is a leakage of potassium ions from bacterial cells [27] phytol, phenol, 2, 4-bis (1-phenylethyl) - which are all have medicinal properties. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies. Mangunwidjaja *et al.* [28] reported the main components of 9,12 octadecadienoic acid, octadec-9-enoic acid and 9,12-actadecadienoic acid present in *Croton tiglium* seed. These compounds were found to have potential antioxidant and anticancer activities. Polyphenol content is maximum in summer in post-reproductive phase. These plants are also rich in minerals like potassium [29].

Pigments are integrally related to the physiological function of leaves. Chlorophylls absorb light energy and transfer it to the photosynthetic apparatus. Carotenoids (yellow pigments) can also contribute energy to the photosynthetic system. However, when incident light energy exceeds that needed for photosynthesis, the carotenoids that compose the xanthophyll cycle dissipate excess energy, thus avoiding damage to the photosynthetic system [30]. Because of the importance of pigments for leaf function, variations in pigment content may provide information concerning the physiological state of leaves. Chlorophyll tends to decline more rapidly than carotenoids when plants are under stress or during leaf senescence [31]. When we investigate pigments in pre-reproductive, reproductive and post-reproductive stages at summer and rainy season as shown in Table 3, it shows variability among chlorophyll a, chlorophyll b, and total chlorophyll. Chlorophyll level is more in pre-reproductive stage during summer while carotenoid and xanthophyll content is more in reproductive phase during summer.

The xanthophyll cycle carotenoids seem to be highly flexible, adjusting rapidly to light conditions [32]. In addition to daily changes, a seasonal pattern has been described, with major changes in the V + A + Z pool content and composition occurring between summer and winter in some evergreen coniferous trees (Adams) carotenoids provide colors to flowers, seeds, fruit, and to some fungi, and color has an important role in reproduction: Coloration attracts animals that disperse pollen, seeds, or spores. Main pigments involved in photosynthesis are chlorophylls and carotenoids. Carotenoids have two well-known functions in photosynthesis: Accessory pigments in light harvesting [33], and as photoprotectors against oxidative damages [34]. It has been proposed

**Table 1: Qualitative phytochemical analysis of *A. ficoidea***

| Phytochemical tests | Source of sample | Solvent used | | | | |
|---|---|---|---|---|---|---|
| | | D.W. | Ether | Chloroform | EtOH | MeOH |
| Alkaloid | | | | | | |
| Marqui's test | Leaf | _ | +++ | +++ | ++ | ++ |
| | Stem | _ | +++ | ++ | + | ++ |
| | Root | _ | +++ | ++ | + | _ |
| Hager's test | Leaf | _ | +++ | ++ | + | _ |
| | Stem | _ | +++ | ++ | + | + |
| | Root | _ | +++ | ++ | + | + |
| Flavonoid | | | | | | |
| Shinoda test | Leaf | ++ | _ | _ | _ | _ |
| | Stem | + | _ | _ | _ | _ |
| | Root | + | _ | _ | _ | _ |
| Ammonia test | Leaf | ++ | _ | _ | _ | _ |
| | Stem | + | _ | _ | _ | _ |
| | Root | + | _ | _ | _ | _ |
| Cardiac glycocides | | | | | | |
| Baljet test | Leaf | + | + | ++ | +++ | +++ |
| | Stem | _ | + | + | ++ | ++ |
| | Root | _ | _ | + | + | + |
| Terpenoid | | | | | | |
| Salkovashi test | Leaf | ++ | _ | _ | ++ | +++ |
| | Stem | + | _ | _ | + | ++ |
| | Root | + | _ | _ | + | ++ |
| Test for tannin | Leaf | +++ | +++ | +++ | +++ | +++ |
| | Stem | +++ | +++ | +++ | +++ | +++ |
| | Root | +++ | +++ | +++ | +++ | +++ |
| Test for reducing sugar | Leaf | _ | _ | +++ | + | + |
| | Stem | _ | _ | +++ | + | + |
| | Root | _ | _ | +++ | + | + |
| Test for anthraquinones | Leaf | _ | +++ | _ | _ | _ |
| | Stem | _ | +++ | _ | _ | _ |
| | Root | _ | +++ | _ | _ | _ |
| Test for saponins | Leaf | +++ | _ | +++ | +++ | + |
| | Stem | +++ | _ | +++ | +++ | + |
| | Root | +++ | _ | +++ | +++ | + |

_: Absent,+: Present in small amount, ++: Moderately present, +++: Present in large amount, *A. ficoidea*: *Alternanthera ficoidea*

**Table 2: GCMS analysis of *A. ficoidea***

| S.No. | Name of the compound | Molecular formula | Molecular weight | Retention time | Area % | Activity |
|---|---|---|---|---|---|---|
| 1 | 3-ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane | $C_{17}H_{50}O_7Si_7$ | 562 | 18.192 | 17.98 | Unknown |
| 2 | 3,7,11,15-tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 26.333 | 19.23 | Antimicrobial |
| 3 | 6,9,12,15-docosatetraenoic acid, methyl ester | $C_{23}H_{38}O_2$ | 346 | 26.525 | 5 | Unknown |
| 4 | 9,12,15-octadecatrienoic acid, methyl ester | $C_{19}H_{32}O_2$ | 292 | 26.658 | 13.43 | Antiinflammatory, nematicide, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, antihistaminic, antiacne, antiarthritic, antieczemic |
| 5 | Phytol | $C_{20}H_{40}O$ | 296 | 26.825 | 44.35 | Antimicrobial, anticancer, cancer preventive, diuretic antiinflammatory |

*A. ficoidea*: *Alternanthera ficoidea*, GCMS: Gas chromatography mass spectrometry

**Table 3: Pigment and polyphenol analysis at three different life stages and two seasons**

| Life stage | Season | Chlorophyll a g/100 g | Chlorophyll b g/100 g | Total chlorophyll g/100 g | Carotenoids g/100 g | $C_{x+c}$ g/100 g | Polyphenols g/100 g |
|---|---|---|---|---|---|---|---|
| Pre reproductive | Rainy | 0.140 | 0.0739 | 0.214 | 0.242 | 0.562 | 4.5 |
| | Summer | 0.157 | 0.112 | 0.269 | 0.314 | 0.952 | 8.08 |
| Reproductive | Rainy | 0.156 | 0.0862 | 0.242 | 0.260 | 0.639 | 5.23 |
| | Summer | 0.134 | 0.121 | 0.255 | 0.311 | 0.709 | 9.16 |
| Post reproductive | Rainy | 0.034 | 0.0263 | 0.060 | 0.070 | 0.230 | 6.08 |
| | Summer | 0.142 | 0.113 | 0.255 | 0.303 | 0.750 | 10.16 |

that carotenoids as light harvesting compounds evolved from anaerobic organisms, then generalized to all of the aerobic photosynthetic organisms. The physical structure of chloroplasts facilitates the transference of energy absorbed by carotenoids to chlorophyll. When leaves are exposed to high illumination, epoxy xanthophyll groups are removed of violaxanthin to initially form antheraxanthin and then zeaxanthin. This is one of the plant protection mechanisms against light damage. The number of carotenoid molecules is higher in sun-exposed leaves than darkness maintained leaves.

This phenomenon is very important, sun-exposed leaves in a fast-growing stage use not more than 50% of absorbed energy during the stage of maximum radiation (midday), and in some species only 10% is used. Thus, 50-90% of absorbed light is in excess and must be eliminated to avoid cellular damage. Xanthophyll cycle is a process that makes the energy dissipation easy and protects the photosynthetic apparatus observed. Moreover, xanthophyll cycle carotenoids are associated with the energy-harvesting complexes photosystem I (PSI) and PSII [35,36]. It was found that higher carotenoid concentrations decreased the peroxide value of soybean oil by quenching singlet oxygen, and it was shown that longer chromophores favor this reaction. Packer [37] evaluated the antioxidant activity of different carotenoids by *in vitro* assays. It was reported that antioxidant activity depends on the used system. Carotenoids, by their antioxidant effect, can show benefits in such diseases; however, this function is not completely demonstrated *in vivo*. Carotenoids have been considered that provide benefits in age-related diseases, against some forms of cancer (in especial lung cancer), strokes, macular degeneration, and cataracts. However, most studies relate dietary components with sickness incidence or symptoms; thus, these studies cannot establish a direct cause-effect relationship. On the other hand, it is clear that carotenoids in association with other components of fruits and vegetables seem to have a protective effect against some chronic diseases and precancerous conditions. Carotenoids protect lab animals of ultraviolet-induced inflammation and a certain type of cancers.

Historically, carotenoid supplementation has been used in the treatment of diseases produced by light sensitivity, which is usually hereditary: 84% of patients with erythropoietic protoporphyria, consuming diets supplemented with β-carotene, increased by a factor of 3 their ability to resist sunlight exposition without presenting symptoms. Furthermore, carotenoids have been used in other photosensitivity diseases: Congenital porphyria, sideroblastic anemia, and have shown only limited success in the treatment of polymorphic light eruption, solar urticaria *Hydroa vacciforme*, *Porphyria variegata, Porphyria cutanea tarda*, or actinic reticuloid [38,39].

The invasive weeds infesting the crop fields and other ecosystems have become a serious problem today. The agroecosystems show an association of various types of native and invasive weeds in crops and barren lands as well as wastelands. To demonstrate the allelopathic potential of selected native and invasive weeds, seed germination bioassays were conducted using the seeds of test crops such as mung and jowar. The protection and conservation of native plant species can be achieved through studies on ecophysiology, allelopathy, and biological invasion of weeds. Scanty research is done on isolation, identification, and characterization of allelochemicals in different native and invasive weeds. Considering these facts, the present investigation was attempted to focus on allelopathic studies of dominant, invasive weed species *A. ficoidea.* The negative impact of higher concentrations of extracts and leachates of different weeds on various crops had been well documented by several workers such as Mallik and Pellissier [40], Djanaguiraman *et al*. [41], Duary [42], Patil *et al*. [43], Ali *et al*. [44], Punjani [45], Travlos and Paspatis [46], Aziz *et al*. [47], and Tanveer *et al*. [48]. They have reported significant inhibition of root and shoot length in *Picea*, wheat, maize, sorghum, rice, blackgram, greengram, soybean, cowpea, cotton and sunflower due to higher concentration treatments of *Vaccinium, Eupatorium, Sesamum, Prosopis, Eucalyptus, Acacia, Grewia, Populus, Casuarina, Amaranthus,* and *Parthenium*.

*A. ficoidea* shows inhibitory effect on germination of mung and jowar. Stress declined the germination and also delayed the germination process. The negative allelopathic activity increases with increase in leaf leachet concentration. Maximum inhibition was shown by 25% leaf leachete from concentration 1%, 2%, 5%, 10%, 15%, and 25% on germination indices such as germination percentage, plumule length, radical length, plumule fresh weight, plumule dry weight, radicle fresh weight, radicle dry weight, meantime germination, MDG, daily germination speed, seedling length vigour index, and seedling weight vigor index also shows allelopathic. As reported above, the phenolic compounds present in the invasive weeds might have caused inhibition of the shoot and root growth in mung bean, because higher concentrations of phenols were detected in the leaves and roots of these invasive weeds. The results on dry biomass of treated seedlings in mung bean and jowar indicate decrease due to the application with higher concentration treatments (Tables 4 and 5). However, Mallik and Pellissier, Duary *et al*. and Djanaguiraman *et al*., Lodha [49] and Ali *et al*. have reported considerable inhibitory effects on dry weight in wheat, rice, black gram, mung bean, cowpea, and *Picea* due to higher concentrations of aqueous leaf extracts and decomposing litter of sunflower, *Eucalyptus, Amaranthus, Parthenium, Sphaeranthus,* sesame, and *Vaccinium,* respectively.

The level of inhibition increased with increase in the concentration of the extracts for all the crops tested. In the present investigation, similar results were obtained regarding the decrease in the dry weight of mung bean and jowar seedlings due to the lower to higher concentration treatment of leaf extracts of *Alternanthera.* This concentration-dependent increase or decrease in dry biomass may be attributed to the explanations given by An *et al*. [50], Orr *et al.* [51] recorded the potential allelopathic influence of two invasive weed species like *Lolium* and *Elaeganus* on native species of *Acer*, *Populus,* and *Platanus.* They found reduction and delay in seedling emergence, root and leaf biomass in treated plants. They explained that it was due to the action of allelochemicals. Swain *et al*. [52] had also reported that the leachates of root, stem, and leaves of *Physalis* inhibited seed germination of *Parthenium* at higher concentrations.

They have claimed that phenolics were responsible for the reduction in seed germination and seedling growth. According to Todaria *et al*. (2005) the tannins, phenols, and other secondary metabolites were responsible for inhibition of seed germination in treated plants. Travlos and Paspatis, Aziz *et al*. and Tanveer *et al*. also observed similar inhibition of germination of their respective test crops. Einhellig (1996) [53], Reigosa *et al*. [54] claimed that the allelochemicals present in the extracts, leachates or residues are responsible for the changes in water relations, membrane permeability and enzymatic activities of protein and carbohydrate metabolism during seed germination process, which determine the success of seed germination. It may be the reasons for the stimulation of seed germination in mung bean when, treated with leaf extracts of *Alternanthera*, *Croton* and *Xanthium* [55].

Lodha and Ali *et al*. have reported considerable inhibitory effects on dry weight in wheat, rice, black gram, mung bean, cowpea and *Picea* due to higher concentrations of aqueous leaf extracts and decomposing litter of sunflower, *Eucalyptus, Amaranthus, Parthenium, Sphaeranthus,* sesame, and *Vaccinium,* respectively. The level of inhibition increased with increase in the concentration of the extracts for all the crops tested. In the present investigation, similar results were obtained regarding the increase or decrease in the dry weight of mung bean and jowar seedlings due to the lower and higher concentration treatment of leaf extracts of *A. ficoidea.* The inhibition or stimulation may be due to various types of allelochemicals such as terpenoids, high phenolic compounds present in the extracts of *Alternanthera.*

Allelopathy may provide alternatives to synthetic herbicides for weed control [56,57]. Laboratory bioassay is the first step to investigate the probable involvement of allelopathy [58] aqueous extract bioassays have been widely employed to evaluate allelopathy of a suspected donor

**Table 4: Allelopathic effect of *A. ficoidea* on mung seeds**

| S.No. | Concentrated of leaf leachet/aspects studied (Mung) | Control D.W. | 1% | 2% | 5% | 10% | 15% | 25% |
|---|---|---|---|---|---|---|---|---|
| 1 | Germinated seeds/10 seeds | 10 | 10 | 9.66 | 10 | 10 | 10 | 8 |
| 2 | Germination (%) | 100 | 100 | 96.6 | 100 | 100 | 100 | 80 |
| 3 | Radicle length (cm) | 14 | 3.73 | 2.76 | 2.43 | 2 | 2 | 1.5 |
| 4 | Plumule length (cm) | 3.56 | 1.73 | 0.96 | 0.15 | 0.23 | 0.1 | - |
| 5 | Radicle fresh weight (g) | 7.62 | 5.14 | 4.61 | 3.7 | 2.336 | 0.2 | 0.05 |
| 6 | Radicle dry weight (g) | 0.885 | 0.524 | 0.594 | 0.0.258 | 0.189 | 0.005 | 0.01 |
| 7 | Plumule fresh weight (g) | 20.70 | 11.76 | 9.05 | 9.04 | 9 | 6.43 | - |
| 8 | Plumule dry weight (g) | 1.19 | 1.74 | 1.70 | 1.87 | 1.184 | 0.73 | - |
| 9 | MTG/7 days | 2 | 2.5 | 2.17 | 5.8 | 5.8 | 5.8 | 0.91 |
| 10 | MDG/20 seeds | 14.28 | 14.28 | 13.80 | 14.28 | 14.28 | 14.28 | 11.42 |
| 11 | DGS | 0.070 | 0.070 | 0.072 | 0.070 | 0.070 | 0.070 | 0.087 |
| 12 | SLVI | 1756 | 446 | 372 | 268 | 223 | 200 | 160 |
| 13 | SWVI | 2832 | 1690 | 1320.37 | 1240 | 1143 | 714 | 408.8 |

SWVI: Seedling weight vigor index, SLVI: Seedling length vigor index, DGS: Daily germination speed, MDG: Mean daily germination, MTG: Mean time germination, *A. ficoidea*: *Alternanthera ficoidea*

**Table 5: Allelopathic effect on Jowar seeds**

| S.No. | Concentrated of leaf leachet/aspects studied (Jowar) | Control D.W. | 1% | 2% | 5% | 10% | 15% | 25% |
|---|---|---|---|---|---|---|---|---|
| 1 | Germinated seeds/10 seeds | 9.66 | 9.66 | 8.66±0.72 | 7.66 | 7.66 | 6.66 | 6.33 |
| 2 | Germination (%) | 96.6 | 96.6 | 86.6 | 76.6 | 76.6 | 66.6 | 63.3 |
| 3 | Radicle length (cm) | 12.83 | 6.06 | 3.16 | 3 | 2.4 | 1.96 | 0.3 |
| 4 | Plumule length (cm) | 6.03 | 2.2 | 1.633 | 1.43 | 1.26 | 1.07 | 0.1 |
| 5 | Radicle fresh weight (g) | 4.53 | 1.76 | 1.34 | 1.14 | 0.82 | 0.469 | 0.09 |
| 6 | Radicle dry weight (g) | 0.568 | 0.391 | 0.28 | 0.18 | 0.177 | 0.147 | 0.05 |
| 7 | Plumule fresh weight (g) | 8.43 | 2.53 | 2.06 | 1.94 | 2.1 | 1.44 | 0.5 |
| 8 | Plumule dry weight (g) | 1.07 | 0.455 | 0.431 | 0.397 | 0.385 | 0.26 | 0.09 |
| 9 | MTG/7 days | 2.06 | 2.85 | 2.69 | 2.56 | 2.90 | 2.65 | 0.6 |
| 10 | MDG/20 seeds | 13.8 | 13.8 | 12.38 | 10.95 | 10.95 | 9.52 | 7.14 |
| 11 | DGS | 0.0724 | 0.072 | 0.080 | 0.091 | 0.0913 | 0.105 | 0.14 |
| 12 | SLVI | 1823.006 | 798.41 | 369.338 | 280.356 | 280.36 | 203.13 | 150 |
| 13 | SWVI | 1296 | 414.67 | 294.64 | 235.928 | 223.67 | 127.206 | 53.77 |

SWVI: Seedling weight vigor index, SLVI: Seedling length vigor index, DGS: Daily germination speed, MDG: Mean daily germination, MTG: Meantime germination species. The speed of germination considers the number of germinated seeds between two exposure times, whereas accumulated germination involves the cumulative number of germinated seeds at each exposure time. Results supported the hypothesis that data interpretations depends on the choice of germination index and also that one index might not be suitable for precise description of allelopathic effects on the germination process. Comparison of different indices may provide better justification. This will contribute in making allelopathy a more precise science.

## CONCLUSION

Investigation of *A. ficoidea* showed presence of large amount of pharmaceutically important phytochemicals like tannins, saponins, phytols, carotenoids xanthophylls and polyphenols. It indicates that this weed can be used in herbal medicines and dietary supplements. Since this weed is showing allelopathy effect it should be eradicated at all the stages from fields before it spreads and occupies the place meant for crop plants.

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