

Allelopathic Effects of Alkaloid Contents of *Hyoscyamus muticus* and *Withania somnifera* on the Germination of *Cichorium intybus* seeds

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ABSTRACT

The aim of current research was to evaluate of the allelopathic effect of aqueous extracts and alkaloid fraction of *Withania somnifera* and *Hyoscyamus muticus* against germination and growth of *Cichorium intybus*, in a laboratory. Alkaloid fraction and aqueous extract of aerial part of *W. somnifera* and *H. muticus* at different concentration, were applied to determine their effect on the seed germination and seedling growth of tested plant under laboratory condition. The germination and growth of *Cichorium intybus* were assessed using growth parameters. The results revealed that all the aqueous extracts markedly suppressed the germination and seedling growth of *Cichorium intybus*. *Withania* extracts showed remarkable effects on germination and the growth of *Cichorium intybus* in comparison to controls. The aqueous extract and alkaloid fraction of *W. somnifera* were more pronounced than *Hyoscyamus muticus* extracts in germination assay. All the aqueous extracts significantly suppressed shoot length, and root length, of *Cichorium intybus*. The inhibition of germination and suppression of growth parameters could be attributed to the presence of active phytochemicals compounds present in the extracts and alkaloid fraction which, subject to GC-MS analysis to study these compound, GC-MS revealed the presence of many active phytochemical compounds, of *W. somnifera* and *H. muticus* extracts (Ferulic acid, Methyl ferulate, Mandolic acid), Scopolamine and atropine are the major compounds in *H. muticus* with concentrations of 13.79% and 3.80% in alkaloid fraction, while in *W. somnifera*, Pterin-6-carboxylic acid are the major compounds with concentration of 2.5 %. The present study concludes that *W. somnifera* and *H. muticus* contain bio-herbicidal compounds in the plant extracts and alkaloid fractions which showed an inhibitory allelopathic effect on the development of wild chicory.

KEY WORDS: ALLELOPATHY, *HYOSCYAMUS muticus* L. , *WATHENIA somnifera*, HARM SEEDS.

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INTRODUCTION

Allelopathy is formulated as any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of agricultural and biological systems. The allelopathic organism releases chemicals that inhibit the growth of a competing organism and thus indirectly prevents it from using common resources. For a long time, it has been recognized that several compounds that are present in the soil in replant situations, actively or passively alter the prevailing soil conditions, inducing a plant population, therefore, due to their allelopathic characteristics, these substances could somehow act as a pesticide (Awatief et al., 2013, Fang and Cheng 2015). *Withania somnifera* is a small shrub to 2 m high, the whole plant is covered with short, fine, silver-grey, branched hairs. The stems are brownish and prostrate to erect, sometimes leafless below.

The leaves are alternate simple, margins entire to slightly wavy, broadly ovate, obovate or oblong, 30–80 mm long and 20–50 mm broad, flowering time is mostly from October to June, while the fruiting time is mostly from October to July (Boulos, 1995). The medicinal importance of *W. somnifera* is due to presence of diverse secondary metabolites. The main chemical constituent of leave of *W. somnifera* are alkaloids (isopelletierine, anaferine, cuseohygrine, anahygrine), steroidal lactones (withanolides withaferins) and saponins (Atta-ur-Rahman 1996). *Withania somnifera* (L.) Dunal screened to investigate the biological activities i.e. antimicrobial, antioxidant, Anti-inflammatory, Anti-aging, Anti-carcinogenic, Cardioprotective, Hypothyroid, Pharmacological activities. The demonstration of broad spectrum of *W. somnifera* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control (Kapoor 2001; Kalpana et al., 2014, Elsharkawy and Shiboop 2017).

Egyptian henbane (*Hyoscyamus muticus*) is one of the most important medicinal plant of the Solanaceae family. It contains valuable tropane alkaloids, hyoscyamin and traces atropine (Lavania, 2005). Tropane alkaloids, especially

hyoscyamin and scopolamine, are widely used in medicine for their mydriatic, antispasmodic, anticholinergic, analgesic and sedative properties. These alkaloids are synthesized in roots and then transported to aerial parts of the plant (Pal Bais et al., 2001). The genus *Hyoscyamus* L. belongs to the tribe Hyoscyameae Miers of Solanaceae family with 18 species all over the world (Yousaf, 2008) and 3 species in Egypt. *Hyoscyamus* species are rich sources of tropane alkaloids, mainly hyoscyamine and scopolamine, Tropane alkaloid have pharmacological and toxicological importance, and are spread in solanaceae family (Kartal et al., 2003). Fatemeh et al., (2012), determined scopolamine and hyoscyamine contents in five *Hyoscyamus* species including *H. niger* L., *H. reticulatus* L., *H. pusillus* L., *H. arachnoideus* Pojark., and *H. kurdicus* Bornm., collected from different geographical origins of North West of Iran by HPLC The range of genetic similarity was obtained between 91.07 and 99.89 within *Hyoscyamus* accessions based on scopolamine and hyoscyamine alkaloids composition.

Allelopathy is the science that studies processes in which secondary metabolites from plants and microorganisms are involved, affecting growth and development of biological systems (Singh et al., 2003) The use of secondary metabolites implicated in allelopathic interactions as sources for news agrochemical models could satisfy the requirements for crop protection and weeds management (Qiming et al., 2016). The aim of current research was to study positive or negative the allopathy of the application different concentration of aqueous extracts and alkaloid fraction of *Withania somnifera* and *Hyoscyamus muticus* against germination and growth of, *Cichorium intybus* in laboratory along with to also study the phytochemical compounds present in plant extract, where the alkaloid fraction was analyzed to understand the role of chemical composition of plants and their allopathic effects.

MATERIAL AND METHODS

Collection and Preparation of Samples: Plants for the study (*Withania somnifera* and *Hyoscyamus muticus*) were collected in June, 2016 from

the wild population in Alamaïn- Wadi El-Natrun Desert Road, Egypt. Seeds of plant weed (*Cichorium intybus*) were collected also collected from Farms of onion in near the plant and kept until germination process. The plant was identified in Desert Research center and the sample was deposited in the Herbarium of Desert Research center.

Laboratory Study: Water Extract preparation:

500 g of dried aerial part of each plants were boiled with 1000 ml distilled water for 2 hour, vigorously stirred and allowed to stand for 24 hours. These were then vigorously re-stirred and filtered into wash bottles until use, thereafter filtered using muslin cloth to obtain a stock solution of 0.5 g/ml concentration. The stock solution was then adjusted accordingly to obtain four different levels concentrations i.e. 25%, 50%, 75% 100%. Distilled water was used as a control. 50 fifty Petri dishes double layered with Whatman No. 1 filter paper were taken and divided into two (2) sets, one set for Aqueous extracts and the other set for alkaloid extracts of roots. The same Experiment was done for each plant. Ten (10) seeds were sown in each Petri dish and moistened with 25 mls of an appropriate water extract of the weed. There were three (3) replications of each treatment (an aqueous and alkaloid extract). Twenty-five (25 mls) of distilled water was applied to the control. Filter paper linings of each Petri dish were moistened daily with an appropriate extract to prevent them drying up before final germination counts (10

days after sowing). The petri dishes were kept in a growth chamber at room temperature until the final germination count. Germination inhibition/stimulation Percentages of inhibition/stimulation effect on seed germination over control were calculated using the formula proposed by Singh and Chaudhary (2011).

Inhibition (-) or stimulation (+) = $\frac{[(\text{Germinated seeds in extracts} - \text{Germinated seed in control}) / \text{Germinated seeds in control}] \times 100}{}$

Measurement of growth parameters: After the completion of seed germination, shoot length was measured using ruler with different days of developmental period. The root length and inhibition percentage of the root length for each seeds of weed was calculated. Germination data obtained were analyzed using analysis (ANOVA) test, the means of treatment were grouped on the basis of (LSD) at the 0.05 probability level. Preparation of Alkaloid fraction: 100 gm of plant powder of each plant under investigation (*W. somnifera* and *H. mutcus*) was separately extracted by refluxing by 70% ethanol three time for 3 hours, filtered of and concentrated by rotary evaporator then suspended in 200 ml water and separated in separating funnel, the filtered aqueous layer was fractionated by chloroform take chloroform fraction and evaporated to obtain (1.8 and 2.6 gm) from two plant ((*W. somnifera* and *H. mutcus*)) respectively, (Elsharkawy and Shiboop 2017). Preliminary Phytochemical screening: Preliminary phytochemical screening

Table 1: Effect of plant extract and alkaloid fraction on the germination *Cichorium intybus*

CONC %	<i>W. somnifera</i>		<i>H. mutcus</i>	
Germination % Plant Extract	Germination % alkaloid fr action	Germination % Water extract %	Germination % alkaloid fraction	
100	0%	0%	0%	0%
75	0%	0%	0%	5%
50	0%	0%	5%	10%
25	10%	5%	10%	15%

Table 2: Phytochemical analysis of *Withania somnifera* and *Hyoscyamus muticus*

Class	<i>W. somnifera</i>	<i>H. muticus</i>
Alkaloids	++	+
Saponins	+++	++
Sterols and steroids	++	+++
Flavonoid	-	+
Tanins	-	-
Anthocyanosids	-	-
Terpene	-	+

of water extracts for each plant for plant secondary metabolite (flavonoids, saponins, alkaloid, terpenes and tannins) was carried out using standard methods (Bao et al., 2005). GC-MS Analysis: Gas chromatography-mass spectroscopy (GC-MS) analysis GC-MS analysis of the plant extracts and fraction were carried out using a Clarus 500 Perkin – elmer (Auto system XL) gas chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.1 spectrometer with an Elite – 1 (100% dimethyl poly siloxane), 30 m × 0.25 mm ID × 1 µm of capillary column. For GC-MS detection, an electron ionization system was

Table 3: Effect of plant extract and alkaloid fraction on the growth parameters of weed *Cichorium intybus*

CONC%	<i>W. somnifera</i>		<i>H. muticus</i>	
	Germination% Water extract	Germination % alkaloid fraction	Germination % Water extract	Germination% alkaloid fraction
Shoot length				
100 %	0	0	0	0
75%	0	0	28%	0
50%	84%	0	83%	0
25%	88%	0	94%	0
Root length				
	Water extract %Germination %	Germination % alkaloid fraction	Water extract Germination %	Germination % alkaloid fraction %
100 %	0	0	0	0
75%	0	0	0	0
50%	68%	0	76%	0
25%	84%	0	88%	0

operated in electron impact mode with ionization system operated in electron impact mode with ionization energy of 70 ev.

RESULTS AND DISCUSSION

The allelopathic effect of plants (*W. somenifera* and *H. muticus*) were carried out by study the effect of aqueous extract and alkaloid fraction of the two plants on the germination of weeds (*Ciocherum*,). The effects of aqueous extract and alkaloid fraction on germination of weeds after 7 days and 15 days in Petri dishes and in pots

respectively are given in Table 1 and Table 2. Our findings demonstrated that two plant *W. somnifera* and *Hyoscyamus muticus* exhibited significant allelopathic activities in all parameters measured (germination, shoot and root length). As shown in Table 1&2, both alkaloid and aqueous extract significantly inhibited germination and reduced shoot and root length of *Cichorium intybus*. The result obtained were concentration dependent as increasing extract concentration significantly inhibited germination of *Cichorium intybus*. The highest inhibitory effect was shown by aqueous extract of *W. somnifera* than *H. muticus*

(100%) of high extract concentration (100%), while the alkaloid fraction of two plants have similar inhibition effect especially at high concentration while at low concentration the inhibition of germination decrease reach to (90% and 85%) for *W. somnifera* and *H. muticus* respectively as illustrated in Table 1. The effect of aqueous extracts and alkaloid fractions of two plants on root and shoot lengths on the studied weed were, like its effects on the germination indices, depending on concentration Table 2,. The root length of *Cichorium intybus*, was significantly reduced by aqueous plant extract and alkaloid fraction, which completely inhibited the root growth. The remaining concentrations of the extract completely inhibited the root growth of weed. In application of low concentration 25% of plant extract and 50% of alkaloid fraction showed significantly increased root length, whereas the higher concentrations induced significant gradual reductions in root length. The effect of extract on shoot length of the weed species exhibited the same pattern as that observed for root growth. At all concentrations, the plant extract inhibited the shoot elongation of, the degree of that inhibition increased gradually in parallel with increasing concentrations of the plant extracts and alkaloid fraction. Phytochemical analysis of

aqueous extract of plants under study revealed the presence of many phytochemical class in two plant alkaloid, saponin, flavonoid and alkaloid as in Table 3.

GC-MS analysis of plants extract showed the presence of many bioactive compounds:

As in Table 4, with their retention index (RI), structure formula and concentration. The results demonstrated the water extract of two plant contain the same phenolic compounds, 4-Hydroxy-3-methoxycinnamic acid (Ferulic acid), Methyl ferulate (Hydroxy-6,7,8-trimethoxy-2,3-dimethyl-4H-chromen-4-one and mono benzylidene- glucose and Mandolic acid, also two plant contain some steroidal compounds, Sarreroside and Ergosta 5,22 dien,3-ol, acetate. There are some compounds are found in *W. somnifera* extract and not detected in *H. muticus*, Corynan-17-ol, 18, 19 di-dehydro 10-methoxy-acetate and 3,9-Epoxy pregnan-14 ol, 20-one,3,11,18-tri-acetoxy. the major compound in *H. muticus* are methyl ferulate (2.5%) and mono benzylidene- glucose (2.80). GC-MS analysis of alkaloid fractions of two plant showed the presence of many bioactive compounds As in Table 5,6 with their retention index (RI), structure formula and concentration. The results demonstrated the

Table 4: Phytochemical analysis of *Withania somnifera* and *Hyoscyamus muticus*

Compounds	<i>Withania somnifera</i>	RI	<i>Hyoscyamus muticus</i>
Methyl ferulate	0.08	604	2.5
4-Hydroxy-3-methoxycinnamic acid	0.06	615	0.08
4-hydroxy-mandonlic acid	0.28	604	0.65
psi.,psi.-Carotene	0.35	501	0.2
Sarreroside	0.1	611	0.66
mono benzylidene- glucose	0.44	521	2.8
Hydroxy-6,7,8-trimethoxy-2,3-dimethyl-4H-chromen-4-one	0.33	420	0.44
Ergosta 5,22 dien, 3-ol, acetate	0.45	632	0.26
Cis Vaccenic acid	0.27	493	-
3,9-Epoxy pregnan-14-ol, 20-one,3,11,18-triacetoxy	1.32	502	-
Corynan-17-ol,18,19-didehydro 10-methoxy, acetate	0.31	404	-
α -D-Glucopyranoside	0.08	586	0.11

alkaloid fraction of two plant contain many same alkaloid compounds Morphinan4,5-epoxy3,6-diol, Strychane, 1-acetyl-20, β -hydroxy-16-methylene, Dasycarpidan1-methanol, Acetate and β -Hydroxyque brachamine with different

concentration. Pterin-6-carboxylic acid are the major compound with concentration (2.5) in plant *Withenia somnifera* where it found in *H. muticus* with low concentration. Scopolamine and atropine are the major compounds in *H.*

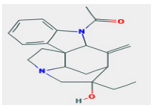
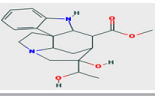
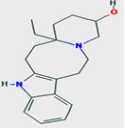
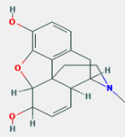
Table 5: GC-MS- analysis of water extract of *Withania somnifera* and *Hyoscyamus muticus*

Compounds	<i>Hyoscyamus muticus</i>	<i>withania somnifera</i>	RI
Methyl ferulate	2.5	0.08	604
4-Hydroxy-3-methoxycinnamic acid	0.08	0.06	615
4-hydroxy-mandonlic acid	0.65	0.28	604
psi.,psi.-Carotene	0.2	0.35	501
Sarrerosite	0.66	0.1	611
mono benzylidene- glucose	2.8	0.44	521
Hydroxy-6,7,8-trimethoxy-2,3-dimethyl-4H-chromen-4-one	0.44	0.33	420
Ergosta 5,22 dien, 3-ol, acetate	0.26	0.45	632
Cis Vaccenic acid	-	0.27	493
3,9-Epoxy pregnan-14-ol, 20-one,3,11,18-triacetoxy	-	1.32	502
Corynan-17-ol,18,19-didehydro10-methoxy, acetate	-	0.31	404
α -D-Glucopyranoside	0.11	0.08	586

Table 6: phytochemical analysis of Alkaloid fraction of *W. somnifera* and *H. muticus*.

Compounds	<i>Hyoscyamus muticus</i>	<i>withania somnifera</i>	RI
Methyl ferulate	2.5	0.08	604
4-Hydroxy-3-methoxycinnamic acid	0.08	0.06	615
4-hydroxy-mandonlic acid	0.65	0.28	604
psi.,psi.-Carotene	0.2	0.35	501
Sarrerosite	0.66	0.1	611
mono benzylidene- glucose	2.8	0.44	521
Hydroxy-6,7,8-trimethoxy-2,3-dimethyl-4H-chromen-4-one	0.44	0.33	420
Ergosta 5,22 dien, 3-ol, acetate	0.26	0.45	632
Cis Vaccenic acid	-	0.27	493
3,9-Epoxy pregnan-14-ol, 20-one,3,11,18-triacetoxy	-	1.32	502
Corynan-17-ol,18,19-didehydro10-methoxy, acetate	-	0.31	404
α -D-Glucopyranoside	0.11	0.08	586

Table 7: Structure of alkaloid fraction in the two plants

Name of compounds	Structure
Strychane, 1-acetyl-20, α -hydroxy-16-methylene	
Curan-17-oic acid, 19,20-dihydroxy-, methyl ester	
β -Hydroxyque brachamin	
Kadain	

muticus with concentration (13.79% and 3.80%) respectively.

W. somnifera and *H. mutcus* are considered indicators allelopathic activity due to their ability of releasing allelopathic substance which has an affect on the germination of other plants. The allelopathic phenomena are found in gardening and agricultural fields since ancient times and this term mean biochemically mediated interaction in plants, many plant were noticed have this phenomena ,so in this study we need to use this phenomena to help us as natural pest side by using plant extract of *W. somnifera* and *H. mutcus* and their alkaloid fraction in germination inhibition of weed Ciochoruim instead of synthetic pest side and try to determine the chemical compound responsible for this inhibition. Secondary metabolites that exert allelopathy can be released in the form of volatile compounds, root exudates, above-ground plant leachates or plant litter [Duke, 2010]. Released allelochemicals are indeed subject to sorption on soil particles as well as chemical and microbial decomposition, (Kaur et al. 2009; Lankau 2010). (Macel et al. 2014), studied the role of many metabolite in six plant in allopathy effect the studied showed that plant chemistry is highly Specific-specific and diverse among

both exotic and native species. Between class of metabolite have a role of allopathic effect are, phenolic compound, sesquiterpene, substituted alkaloid and flavonde in between phenolic compound have allopathy effect cinnamic acid derivatives (Lankau, 2010.), this are agree with our results which demonstrate the presence of cinnamic acid derivatives and methyl ferulate as constituents of two plant extracts. Also alkaloid compounds present in plant extract have a role in germination inhibition.

CONCLUSION

It is concluded that the aqueous extract and alkaloid fraction of *Withania somnifera* and *H. mutcus* showed an inhibitory allelopathic effect on the germination of wild chicory and the inhibitory effect depended on the concentration. Phytochemical analysis showed the plant contain different class of phytochemical compounds GC-MS analysis of aqueous extract revealed the presence of hydroxyl cinnamic acid and methyl ferulate and other phenolic compound which may have a major role in germination inhibition also alkaloid fraction have many compounds which consider as allelochemical finally we can recommended by use these plant extracts as a pest side after further studies

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CONFLICT of INTEREST

The author declare any conflict of interest

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