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EFFECTS OF BIOCHEMICALS OF *ARTEMISIA ANNUA* IN PLANTS

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Artemisia annua L. a plant of temperate agroclimate and native of China has been used as an anti-malarial drug specially in cerebral malaria for centuries and herbicidal compound artemisin (sesquiterpene lactone).To find out more effective herbicidal compounds than artemisin and its related compounds,the well known derivative of *A.annua i.e.* artemisinic acid and arteannuin B and semisynthetic derivative of artemisin compound *i.e.* arteether, followed the laboratory testings for their role in seed germination and seedling growth in some of the monocot and dicot plants including *Artemisia annua, Lactua sativa* (Asteraceae), *Raphanus sativus* (Brassicaceae), *Portulaca oleracea* (Portulacaceae), *Amaranthus blitun* (Amaranthaceae), *Secale cereale, Hordeum vulgare* (Poaceae).The biochemical compound namely arteether was found to be very effective with respect to all the tested chemical compouds.This compound produced 50% inhibitory effects in the root growth at the rate of 1 ppm in case of *Secale cereale, Hordeum vulgare* and shoot growth in case of *Lactua sativa*.In *Portulaca oleracea* arteether caused inhibitory effect both in case of shoot and root development at the same rate of 1 ppm .This compound arteether also showed retarded growth of seedling in case of *Artemisia annua* and *Amaranthus blitun* at the rate of 10 ppm.But at this particular concentration rate it has shown shoot growth retardation in case of *Secale cereale* and *Hordeum vulgare*. Chemical compound named as artemisin also showed similar effect but at lower activity rate with respect to that of arteether in most of the test plant samples. At a lowest concentration all the plant samples of *A. annua* chemical compounds showed seedling growth .Growth and developmental activity of arteannuin B and artemisinic acid found to be more effective with respect to that of arteether and artemisinin.

Keywords: Arteannuin B; Arteether; *Artemisia annua;* Artemisinin; Artemisinic Acid; Asteraceae.

Introduction

The herb *Artemisia annua* is a native of China and now grow in many countries such as Australia, Argentina, Bulgaria, France, Hungary, Italy, Spain and United States.In India,this herb is often cultivated in the Himalayan regions.The plant is totally acclimatized to North Indian conditions. The drug *A.annua* has been used as an antimalarial, specially in cerebral malaria for centuries[1].The plant is good stomachic, diuretic,given in jaundice patients, skin diseases and is effective in diabetes.Leaves yield an essential oil which is used in ointments[2]. In Chinese literature,the decoction from this plant has been mentioned as a remedy for chills and fever.The earliest report on the use of extract of *A.annua* was in the "Recipes for fifty two preparations" that was found in" Mawanghdui Han Dynasty Tombs" dating to 168 BC, which recommended its use for hemorrhoids. Anti-tumorous, cytotoxic, chloretic, anti complementary, insecticidal, anti microbial, antihelmintic, anti fertility, anti diabetic,anti convulsant and other activities have been reported for the flavonoids of this genus. *A.annua* is the natural source of artemisinin, a sesquiterpene lactone with an internal peroxide linkage[3].

Plants and their residues commonly produce inhibitory or stimulatory effects on the vegetation which grows nearby through release of their chemicals to the atmosphere and / or soil. This chemical mechanism of plant to plant interference is known as allelopathy and the phytochemicals that mediate plant to plant interaction are known as the allelochemicals[4,5]. Each of the interacting plants may produce one or more allelochemicals. The effects of allelochemicals produced by the plants growing together determine the vegetation patterns and the levels of effects of the activity on crops and weeds[6-9]. A variety of secondary metabolites have been identified as allelochemicals in the plants that have been studied for their allelopathy. Simple phenolic acids, coumarins,

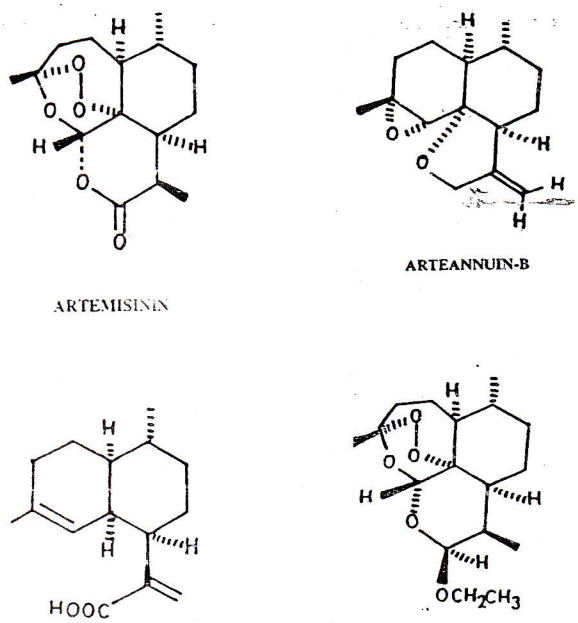

Fig.1. Structures of artemisinin and its related compounds.

terpenoids, flavonoids, alkaloids, cynogenic glycosides and glucosinolates were reported to be the most common groups of allelochemicals[5,7,10,11].

In several plants of the family asteraceae, sesquiterpene lactones and monoterpenes that are commonly found in the glandular trichomes of the plants serving as a chemical defense mechanism against herbivores have also been identified to be allelochemicals. *Artemisia* is the largest genus of the Asteraceae belonging to the tribe anthemidiae, is well known for such bitter and toxic substances[12,13]. *A annua*, an important medicinal plant of Chinese origin, has been receiving increased attention on account of its antimalarial and phytotoxic properties. The leaves and flowers of this plant contain artemisinin[14], an endoperoxide sesquiterpene lactone that was found effective against chloroquine and mefloquine resistant strains of plasmodium falciparum, the pathogen responsible, has been reported to inhibit seed germination and seedling growth of several monocot and dicotyledonous plants. In addition to the artemisinin, *A. annua* contains some related sesquiterpene lactones, which are also reported to have a variety of biological properties including growth regulatory and allelopathic activities[15-17]. Arteether, obtained by the etherification of dihydroartemisinin a reduction product of artemisinin, has been proved to be a far more active, against malarial parasites than artemisinin[2,18,19]. If the mechanisms of phytotoxic and antiparasitic actions are similar, then arteether should prove more phytotoxic than artemisinin. This question has been examined in the present study where the phytotoxicity of arteether has been compared with that of artemisinin, artemisinic acid and arteannuin B, using seed germination and seedling growth, assays on certain monocot and dicotyledonous plants.

Materials and Methods

Chemicals used: Pure compounds used in this investigaitoan were artemisinin, artemisinic acid, arteannuin B and arteether (Fig. 1). For isolation of former three compounds, aerial parts of the plant were extracted with n-hexane and concentrated under reduced pressure and the concentrate was defatted and subjected to column chromatography over silica gel, eluting with hexane, 5% EtoAc-hexane, 10% EtoAc-hexane and 15% EtoAc-hexane. The 5, 10 and 15% fraction were rich in artemisinic acid, artemisinin and arteannuin B, respectively. The entire fraction on crystallization afforded pure crystalline compounds; which were characterized by mp, mmp, IR, NMR and MS methods. Arteether was prepared by the etherification of dihydroartemisinin, a reduction product of artemisinin. Test solutions were freshly prepared by dissolving the compounds in 100% spectral grade acetone as stock solution. Final concentrations were obtained by dilution with distilled water. The control treatment used was 20μl of acetone, which is the highest concentration in the test solutions used.

Bioassay: Seeds of *Hordeum vulgare, Raphanus sativus, Portulaca oleracea* and *Amaranthus blitum* were purchased from a seed store. Seeds of *Lactuca sativa, Secale cereale* and *Artemisia annua* were collected from their accessions grown at GKVK, CIMAP, and IIHR campuses. To conduct an assay, twenty seeds of a species were placed on Whatman filter paper moistened with a concentration (200, 100, 10, 1.0, 0.1 ppm) of the test solution in 9 cm diameter Petri dishes. The Petri dishes were then incubated in a germinator at 25°C with 90% humidity and 4th light / day for nine days. Germination and seedling growth were scored on the third, sixth and ninth days. Assays on all the treatments were conducted together. The experiments were repeated many times to confirm the best possible results.

Results and Discussion

Effect of biochemical compounds (Artemisinic acid, Arteannuin- B, Artemisinin, and Arteether) *on the germination of seeds* : The effect of artemisinic acid, arteannuin B, artemisinin and arteether individually, on seed germination of several monocot and dicotyledonous species is shown in Table 1.

Artemisinic acid inhibited seed germination in *P. oleracea* and *L. sativa* at 10 ppm. In *H. vulgare* and *A. blitun* it produced inhibitory effect at 100 ppm. Artemisinic acid was not very effective in *A. annua*. In *S. cereal* and *R. Sativus* it did not show inhibitory effect even at 200 ppm. In *A. blitun*, it promoted seed germination at 0.1 to 10.0 ppm.

An inhibitory effect of arteannuin B was not observed in any of the examined species below 10 ppm. It promoted seed germination in *A. blitun* up to 1 ppm. At 100 ppm, it produced an inhibitory effect on germination of *S. cereal* and *L. sativa* seeds. The rest of the examined species were affected only at 200 ppm, while *R. sativus* was resistant even at this concentration. Seeds of *A. blitun* did not germinate at 200 ppm.

Artemisinin at 0.1 ppm promoted seed germination in *P. oleracea* and *A. blitun*. It inhibited germination of *L. sativa* at 10 ppm and in *S. cereale* and *A. blitun* at 100 ppm. The rest of the species, except *A. annua*, showed inhibition at 200 ppm. Seeds of *P. oleracea* and *A. blitun* did not germinate at 200 ppm.

Inhibition of seed germination by arteether was not observed in any of the seeds examined below 10 ppm. However, arteether promoted germination in *L. sativa* and *A. blitun* at 0.1 and 1.0 ppm, respectively. Again in *A. annua*, arteether was more effective than artemisinin. Arteether inhibited seed germination in *L. sativa* at 100 ppm and in the rest of the species at 200 ppm. *R. sativus* was not affected at this concentration. Seeds of *P. oleracea* and *A. blitun* did not germinate at 200 ppm.

Effect of biochemical compounds (Artemisinic acid, Arteannuin- B, Artemisinin, and Arteether) *on the shoot growth of the plant:* The effect of artemisinic acid, arteannuin B, artemisinin and arteether on shoot growth of seedling is shown in Table 2.

Artemisinic acid at lower concentrations promoted shoot growth of seedlings in all the examined dicotyledonous species. In monocotyledonous plants it was neighter an effective growth promoter nor inhibitor. Artemisinic acid inhibited shoot growth in *P. oleracea* and *L. sativa* at 10ppm; in *A. annua* and *A. blitun* at 100 ppm. The seedlings of all the species examined tolerated artemisinic acid even at 200 ppm concentration.

Growth promoting activity of arteannuin B was observed in both monocot and dicotyledonous species at lower concentrations. It promoted shoot growth of *H. vulgare, A. annua, R. sativus* and *A. blitun*. It inhibitted shoot growth at 100 ppm in *S. cereale* and *H. vulgare. In S. cereale* and dicotyledonous species excepting *R. sativus*, the compound was effective at 200 ppm.

Artemisinin promoted shoot growth in *H. vulgare, P. oleracea* and *L. sativa* at 1 ppm or below. In *A. annua*, artemisinin promoted growth up to 10 ppm and there was no significant inhibitory effect even at 200 ppm. The inhibitory effect of artemisinin above the 10 ppm concentration was more pronounced in monocotyledonous species than dicotyledonous species, in the former it produced discoloration of leaves at 10 ppm and killed the seedlings at 100 ppm. The seedlings of *A. blitun* were killed at 100 ppm and rest of the examined species except *A. annua* at 200 ppm.

Arteether was found to be a very effective growth inhibitor. It inhibited the shoot growth of *P. oleracea* and *L. sativa* seedlings at 1 ppm and in most of the species examined at 10 ppm. In *R. sativus*, however, it was effective at 100 ppm. Arteether, like artemisinin, caused discoloration of leaves in the monocotyledonous species at 10 ppm; to those of *A. annua* and *A. blitum* at 10 ppm and that of *R. sativus* and *L. sativa* at 100 ppm. The seedlings of the monocotyledonous species were killed by arteether at 200 ppm concentration.

Effect of biochemical compounds (Artemisinic acid, Arteannuin- B, Artemisinin, Arteether) *on the growth of roots :* The effects of the *A. annua* compounds on root growth are shown in Table 3.

Artemisinic acid, except in *A. annua*, promoted root growth in all the examined monocot and dicotyledonous plants at lower concentrations. Growth promotion activity was more pronounced in the monocotyledonous species than in dicotyledons. In *P. oleracea* and *A. blitum*, artemisinic acid inhibited root growth at 100 ppm, and in most of the examined seedlings at 200 ppm. Against *S. cereale*, artemisinic acid was not very effective.

Arteannuin B promoted root growth below 1 ppm concentration in *H. vulgare, R. blitum* and *L.sativa*. The growth promotion activity of arteether was very high in *H. vulgare*. Arteannuim B reared the root growth at 100 ppm in most of the examined seedlings except in those of *R. sativus* and *L. sativa*, where the compound was active only at concentrations of 200 ppm or more.

Artemisinin at its low concentrations promoted root growth in the seedling of *S. cereale, P. oleracea* and *A. blitum*. But it was a very effective root growth inhibitor in both of the monocotyledonous species examined (*S. cereale and H. vulgare*) at 1ppm; in *A.annua* and *A.blitun* at 10ppm; and in *R. sativa* and *L.sativa* at 100 pp. The seedlings of the monocotyledonous species were killed by arteether at the 200ppm concentration.

It was observed that artemisinin inhibited the

Table 1. Effect of biochemical compounds on the germination of seeds in case of monocot and dicot plants.

Compound	Concentration (ppm)	Germination (%) of seeds in relation to control on 9th day after sowing in the plant.						
		Secale cereale	*Hordeum vulgare*	*Artemisia annua*	*Raphanus sativus*	*Portulaca oleracea*	*Amaranthus blitun*	*Lactuca sativa*
Artemisinic acid	0.1	100	90	100	100	83	100	86
	1.0	100	70	90	100	67	150	57
	10.0	100	57	88	100	33	150	42
	100.0	100	43	85	100	33	17	29
	200.0	100	29	65	100	33	17	29
Arteannuin-B	0.1	100	78	100	100	100	100	86
	1.0	100	89	100	100	100	143	71
	10.0	75	78	98	100	100	100	57
	100.0	50	66	95	100	83	86	43
	200.0	13	14	50	100	0	0	29
Artemisinin	0.1	100	89	95	100	114	129	86
	1.0	88	89	90	100	100	100	71
	10.0	75	78	90	100	86	71	29
	100.0	50	78	85	100	71	43	15
	200.0	25	14	65	30	0	0	12
Arteether	0.1	100	89	80	100	100	100	129
	1.0	100	89	80	100	100	100	129
	10.0	75	78	75	100	89	166	86
	100.0	75	67	65	100	67	67	43
	200.0	50	29	40	90	0	0	43

Table 2. Effect of biochemical compounds on the shoot growth in case of monocot and dicot plant seedlings.

Compound	Concentration (ppm)	Present shoot growth of seedling in relation to control on 9th day after sowing.						
		Secale cereale	*Hordeum vulgare*	*Artemisia annua*	*Raphanus sativus*	*Portulaca oleracea*	*Amaranthus blitun*	*Lactuca sativa*
Artemisinic acid	0.1	91	81	114	98	126	171	161
	1.0	93	86	102	105	112	160	104
	10.0	83	79	92	101	52	137	63
	100.0	79	54	72	73	9	119	22
	200.0	78	51	50	60	9	48	15[b]
Arteannuin B	0.1	88	123	131	109	75	162	74
	1.0	76	92	111	114	99	167	135
	10.0	60	69	106	106	136	170	52
	100.0	44	46	100	82	87	75	54
	200.0	01	44	24	78	15	0	43[b]
Artemisinin	0.1	103	108	107	95	123	154	161
	1.0	96	110	114	92	150	57	104
	10.0	43[a]	69[a]	105	87	59	64	63
	100.0	22[b]	43[b]	74	61	42	40[b]	22
	200.0	13[b]	02[b]	59	22[b]	0	0	15[b]
Arteether	0.1	91	89	94	92	159	178	108
	1.0	75	114	72	82	47[b]	105	48
	10.0	46[a]	46[a]	31[b]	67	37[b]	43[b]	42
	100.0	19[a]	41[a]	24[b]	53[b]	9[b]	0	43[b]
	200.0	04[b]	01[b]	20[b]	40[b]	0	0	13[b]

a: Discoloration of leaves were noted carefully.
b: Seedlings showed the sign of death.

Table 3. Effect of biochemical compounds on the growth of roots in case of monocot and dicot plant seedlings.

Compound	Concentration (ppm)	Present root growth of seedling in relation to control on 9th day after sowing.						
		Secale cereale	*Hordeum vulgare*	*Artemisia annua*	*Raphanus sativus*	*Portulaca oleracea*	*Amaranthus blitun*	*Lactuca sativa*
Artemisinic acid	0.1	96	139	82	144	117	147	91
	1.0	160	147	68	93	84	115	133
	10.0	100	185	62	92	51	82	81
	100.0	58	115	52	56	20	43	69
	200.0	53	45	19	23	18	17	8[b]
Arteannuin B	0.1	85	246	74	99	54	138	101
	1.0	61	135	66	120	76	138	135
	10.0	52	92	57	84	54	80	61
	100.0	10	50	48	62	16	29	53
	200.0	01[a]	44	10[a]	10	0	0	52[a]
Artemisinin	0.1	121	67	66	99	149	159	89
	1.0	34	44	64	87	153	89	124
	10.0	17	44	58	86	94	68	90
	100.0	03[a]	6[a]	44	62	36	43[a]	57
	200.0	02[a]	12[a]	23	5[a]	0	0	13[a]
Arteether	0.1	62	90	146	62	118	188	39
	1.0	38	46	100	109	51[a]	80	37
	10.0	5	16	19[a]	92	53[a]	43[a]	36
	100.0	3[a]	6	13[a]	36[a]	26[a]	39[a]	35[a]
	200.0	2[a]	3[a]	10[a]	11[a]	0	0	13[a]

a= Seedlings showed the sign of death.

germination in *L. sativa* and *A. annua* and growth of seedlings of *L. sativa, Amarnathus retroflexus, Ipomoea lacunose, A. annua* and *P. oleracea* at 33μM (9.24ppm)[16]. At this concentration, artemisinin had no effect in their study on the growth of *A. blitun* and *Sorghum biocolor.* It was further observed that arteannuin B and quinghao acid (artemisinic acid) at 33μM (8.1 and 7.5 ppm respectively) inhibited the germination but had little effect on the growth of the surviving *L. sativa* seedlings[16]. Artemisinin is reported to be phytotoxic to both terrestrial and aquatic plants and completely inhibited root induction in the examined species, arteannuic acid (artemisinic acid) and arteannuin B, were less toxic and stimulated the root induction[15]. The effect of artemisinin and arteannuic acid was also evaluated on the physiology of the auatic plant *Lemma minor*. Artemisinin at 5μM inhibited the food production. Chlorophyll content was also reduced by artemisinin at 2.5μM.. Arteannuic acid at 10μM was less active. Artemisinin at 1μM reduced respiration (39%). Arteannuic acid had no significant effect on photosynthesis or respiration at the levels tested[17]. Thus, artemisinin was the most active growth inhibiting phytotoxic compound isolated so far from *A. annua* plants. In the present study also artemisinin was found to be more phytotoxic than arteannuin B and artemisinic acid. However, arteether proved to be more phytotoxic than artemisinin. All the compounds are found to be better inhibitors of root growth, than shoot growth.

The present work has led to the identification of *R. sativus* and *A. annua* plants to be highly tolerant to the phytotoxic effects of artemisinin related compounds. Artemisinin was reported to be autotoxic to *A. annua* at 33μM[16]. However, we find arteether more toxic to *A. annua* seedlings than artemisinin. Artemisinin and arteether were both observed to cause discoloration of the leaves of the monocotyledonous plants. Partial disintegration of the chloroplast might have been the cause of leaf discoloration[17].Accordingly in the *R. sativus* chloroplast integrity must be immune to the detrimental action of artemisinin or arteether.

Root growth in *A. annua* was more susceptible to the action of artemisinin and arteether than the shoot. This might be an indication of the presence of a protective mechanism in the *A. annua* shoot where artemisinin is synthesized. It would be interesting to examine the nature of the toleration mechanism in *R. sativus* which belongs to a different family.

An interesting observation was the ability of the artemisinin related compound of *A. annua* to promote seedling growth at lower concentration. The growth promoting activity in artemisinin acid was highest followed by arteannuin B, artemisinin and arteether.

The artemisinin compounds studied here were found to possess both plant growth promoting and cell killing activities, depending on their concentration of use. The antimalarial activity of artemisinin has been largely attributed to its endoperoxide moiety. It is reported that free radicals arising from this moiety cause death of malarial parasites which infect red blood cells[20]. Arteether has been shown to act on the parasite by the same mechanism as artemisinin[18]. It is inferred that artemisinin acid and arteannuin B are ineffective because the endoperoxide moiety is absent in their structure (Fig. 1). The relative phytotoxicities of these compounds indicate that endoperoxide moiety must be involved in the plant cell killing action of artemisinin and arteether. There must be additional sites of activity on artemisinin and related compound as artemisinic acid and arteannuin B also have some phytotxicity and all the compounds are able to promote seedlings growth at lower concentration.

Conclusion and Implication: Artemisinin at its low concentrations promoted root growth in the seedling of *S. cereale, P. oleracea* and *A. blitun*. But it was a very effective root growth inhibitor in both the monocotyledonous species examined *CS. cereale* and *H. vulgare*) at 1ppm; in *A.annua* and *A.blitun* at 10ppm; and in *R. sativus* and *L.sativa* at 100 pp. The seedling of the monocotyledonous species was killed by arteether at the 200 ppm concentration. Arteether promoted the shoot growth in *P.oleracea, A. blitun* and *L.sativa* seedling at 0.1ppm.

Root growth in *A. annua* was more susceptible to the action of artemisinin and arteether than the shoot. This might be an indication of the presence of a protective mechanism in the *A. annua* shoot where artemisinin is synthesized.

An interesting observation was the ability of the artemisinin related compound of *A. annua* to promote seedling growth at lower concentration. The growth promoting activity in artemisinin acid was highest followed by arteannuin B, artemisinin and arteether.

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