Degrading phenolic compounds and exhausting food reserves stored in the tubers of *Cyperus rotundus* L. with hydrolytic enzyme

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ABSTRACT

***Cyperus rotundus* is one of the world worst weeds causes hundred per cent yield loss at times. Killing the mother tubers with the foliar applied herbicide prevent the translocation of herbicides to the secondary and tertiary tubers. Further the tubers can survive during adverse weather condition due to the presence of phenolic compounds and the food reserve. Hence an experiment was carried out with hydrolytic enzyme to degrade the phenolic compounds (germination inhibitor), to stimulate the germination and exhaust the food reserve in the tubers of weeds to kill before emerging. In the first attempt four different concentration of alpha amylase (50, 100, 150 and 200 ppm) and three different durations (6, 12 and 24 hours) were evaluated. In the second attempt six different concentrations (250, 500, 750, 1000 and 1250 ppm) of alpha amylase were tested for 72 hours. The biochemical parameters namely starch (Anthrone method), amylose (Rapid method) and phenols (Spectrophotometric method) were recorded at hourly intervals and viability of the tubers was tested following tetrazolium test. In the present study, it is observed that treating the tubers of purple nut sedge with alpha-amylase at 200 ppm recorded the minimum content of starch (50 mg g-1) and maximum content of amylose (39 mg g-1) after 24 hours of soaking. Alpha-amylase acts on starch and breaks into glucose molecules, which may be due to the hydrolysis of starch to glucose and maltose by the alpha amylase enzyme. Soaking of alpha-amylase enzyme at 1250 ppm has recorded the minimum content of starch of 32 and 39 mg g-1 in the whole as well as cut tubers, respectively. Thus the hydrolytic enzyme, alpha-amylase effectively degraded the food reserve leading to death of tubers before emerging out.**

Key words: alpha amylase, germination, phenols, purple nut sedge, starch, tubers, weeds

Purple *(C. rotundus*) and yellow nutsedge (*C. esculentus*) are troublesome perennial weeds in many parts of the world (Holm *et al*., 1977). They propagate primarily by tubers that can sprout repeatedly often producing more than one shoot at a time (Bendixen, 1973; Stoller *et al*., 1972; Thullen and Keeley, 1979). Most tubers sprout in spring, but some tubers remain dormant for more than 3 years (Stoller and Sweet, 1987) and certain yellow nutsedge tubers remain dormant up to 10 years (Neal, 1995). Consequently, nutsedge control strategies must include a long-term commitment to prevent the new tuber formation. Although both purple and yellow nutsedge produce viable seeds, they are insignificant for propagation because of poor seedling vigour (Lapham and Drennan, 1990; Stoller and Sweet, 1987). Agronomical practices like crop rotation and cultivation, soil desiccation did not show efficient control of this weed. Similarly field plough, hand weeding, hoeing also could not control sufficiently (Horowitz, 1972). Complete control of these weeds is not possible because of their growing nature. Bulbs and rhizomes are located in the underneath soil, chemical sprayed reaches only the above ground parts. None of the herbicides reaches the rhizome network. So they are still alive and act as a source for new plant in the next season. The effective herbicide treatments must outlast the tuber's ability to resprout *i.e* the chemical must remain active for 10 to 12 weeks (Lanini, 1987). Most of the soil applied herbicides get degraded quickly due to one or the other factor.

The complete control or inhibition of nutsedge is possible by altering the physiology of the propagating materials. The tubers produced by the weeds are entering into the dormancy during the unfavourable conditions. The presence of phenols in the tubers inhibits the sprouting of buds during unfavourable situation (Jangaard *et al.,* 1971) and during favourable conditions one or two buds sprout others remain in dormant condition. Under this situation, new strategies have to be designed to break the dormancy and killing the sprouted tubers chemically or desiccating the germinated tubers culturally, degrading the starch, the food reserve in the tubers also helps to reduce the incidence and carry over to the next season. Thus germinated tubers could be easily killed with the herbicides available already. In this study hydrolytic enzyme was used to degrade the germinating inhibitor (phenolic compounds) to induce the germination of buds at once present in the tubers and exhaust the food reserve (starch).

MATERIALS AND METHODS

Lab experiment was conducted in the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, during 2011-12.Three sets of laboratory experiments were conducted to optimize the concentration of alpha amylase and duration with whole and cut tubers. In the first experiment four different concentration of alpha amylase (50, 100, 150 and 200 ppm) and three different durations (6, 12 and 24 hours) were replicated thrice with whole tubers in the Factorial Complete Randomised Block Design. In the second and third experiment six different concentrations (250, 500, 750, 1000 and 1250 ppm) of alpha amylase were tested for 72 hours with whole and cut tubers in a Complete Randomized Block Design with three replications separately. The biochemical components and viability of the purple tubers were analyzed.

RESULTS AND DISCUSSION

The present study was conducted to evaluate the effect of alpha-amylase on the purple nutsedge tuber's biochemical composition and viability. The tuber composition was determined after treating with various concentrations of alpha-amylase at different duration levels.

Effect of Alpha-amylase enzyme on the biochemical components of starch, amylose and phenol content in the purple nut sedge tuber

Alpha-Amylase is an enzyme that hydrolyses alpha-bonds of large alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. Starch is very important molecule for energy storage in nature and is usually insoluble in cold water.

Table 1. Effect of Alpha-amylase enzyme on total starch (mg g-1) content in the tubers of purple nutsedge

Conc. (ppm)	Duration			
	6 hrs	**12 hrs**	**24 hrs**	**Mean**
T_1-Control	72	70	65	60
T_2-50	67	65	54	56
T_3-100	61	57	47	58
T_4-150	58	53	38	53
T_5-200	51	45	27	50
Mean	68	55	43	
	T	D	T×D	
SE.d	0.53	0.41	0.92	
CD (P = 0.05)	1.09	0.84	1.88	

Table 2. Effect of Alpha-amylase on amylose (mg g-1) content in the tubers of purple nut sedge

Conc. (ppm)	Duration			
	6 hrs	**12 hrs**	**24 hrs**	**Mean**
T_1-Control	19	23	29	30
T_2-50	22	27	32	33
T_3-100	28	33	38	34
T_4-150	35	39	47	35
T_5-200	41	46	55	39
Mean	24	33	46	
	T	D	T×D	
SE.d	0.33	0.26	0.58	
CD (P = 0.05)	0.68	0.53	1.18	

In plants, starch is made in specialized cellular organs called amyloplast. The starch forms granules with consistent size and shape, depending on the plant that made them. Degrading the food reserve present in the propagating materials like tubers may lead to death of tubers or reduce the multiplication rate.

A highly significant difference on the starch content was observed with the different

concentrations of alpha-amylase under different duration of treatment. As the concentration increases, there was reduction in the content of starch stored in the purple nut sedge tubers. In the present study treating the tubers with alpha-amylase at the rate of 200 ppm recorded the minimum content of starch (50 mg g^{-1}) and maximum content of amylose (39 mg g^{-1}) 24 hours after soaking (Table. 1, 2). Alpha-amylase act on starch and break them up into simple polymers consist of sugars and glucose (Poonam and David, 2000; Pandey *et al*., 2000). This may be due to the hydrolysation of starch to glucose and maltose by the alpha amylase enzyme. Increased hydrolyzation was recorded with the long hours of soaking. This is in line with findings of Elif Sarikaya *et al*. (2000). They have opined that a long hydrolysis period of 24 to 72 hours is required to degrade the starch content.

It was observed that as the concentration of alpha-amylase enzyme increased, starch degradation also gets increased. The alpha-amylase enzyme at 1250 ppm has recorded the minimum content of starch (32 and 39 mg g^{-1}) followed by 1000 ppm (44 and 48 mg g^{-1}) in the whole as well as cut tubers, respectively. As a result of degradation of tubers due to the catalytic activity of alpha-amylase, the resultant product of amylose content gets increased in the tubers. It is evident from the results that treating the purple nutsedge tubers with 1250 ppm of alpha-amylase recorded the maximum content of amylose (63 and 67 mg g^{-1}) followed by 1000 ppm (51 and 59 mg g^{-1}), respectively in the uncut and cut tubers.

Further the hydrolytic enzyme alpha-amylase similarly degraded the total phenol content of the tubers. Application of alpha-amylase at the rate of 1250 ppm has recorded the minimum content of phenol (5.0 and 4.0 mg g^{-1}) followed by 1000 ppm (5.9 and 4.7 mg g^{-1}) in the whole and cut tubers, respectively.

The hydrolytic enzyme could be used to exhaust the food reserve present in the tubers to prevent the multiplication rate of tubers.Based on the results of previous experiment, higher concentration of alpha-amylase was tried with longitudinally cut tubers to degrade the entire starch stored in the tubers. Use of higher concentration of alpha-amylase accelerated the degradation process. It was evident from the results that alpha-amylase enzyme at 1250 ppm recorded the minimum content of starch (32 mg g^{-1}), maximum content of amylose (63 mg g^{-1}) and minimum content of phenol (5 mg g^{-1}) in whole tuber compared to cut tubers (39 mg g^{-1}, 67 mg g^{-1}, 4 mg g^{-1}) at the same concentration (Table. 3). Jensen and Jorgen (1992) stated that the soluble starch, amylose and amylopectin were completely degraded by the alpha-amylase at higher concentration. When the whole tubers (uncut) were subjected to the same concentration of enzymatic solution, comparable quantities of the biochemical components were observed as that of cut tubers.

Table 3. Effect of Alpha-amylase enzyme on the biochemical components of whole and cut purple nutsedge tubers three days after soaking

Conc. (ppm)	Starch (mg g^{-1})		Amylose (mg g^{-1})		Phenols (mg g^{-1})	
	WT	**CT**	**WT**	**CT**	**WT**	**CT**
T_1-Control	90	110	22	25	8.8	8.8
T_2-250	75	79	29	33	7.9	7.6
T_3-500	63	64	35	45	7.1	6.2
T_4-750	51	59	42	51	6.6	5.0
T_5-1000	44	48	51	59	5.9	4.7
T_6-1250	32	39	63	67	5.0	4.0
Mean	59	67	40	47	6.8	6.0
SE.d	1.01	1.14	0.69	0.79	0.11	0.10
CD (P = 0.05)	2.21	2.50	1.51	1.73	0.24	0.22

WT – Whole Tuber; CT – Cut Tuber

CONCLUSION

It is conclude that soaking the tubers with alpha amylase with minimum concentration (200 ppm) recorded the minimum content of starch (27mg g^{-1}) and maximum content of amylose (55 mg g^{-1}). At the higher concentration (1250 ppm), the least content of starch (32 mg g^{-1}) and maximum content of amylose (63 mg g^{-1}) with minimum content of phenol (5 mg g^{-1}) were recorded. The hydrolytic enzyme could be used to exhaust the food reserve present in the tubers to prevent the multiplication rate of tubers. The degree of starch

also gets increased due to the continuous enzymatic activities while extending the soaking duration.

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