EFFECT OF *CANNABIS SATIVA* L. EXTRACT TO OXIDATIVE STRESS OF *Sorghum halepense* L.

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Abstract

The growing desire to find new biopesticides has put allelopathy at center of research interest. Aim of this study was to investigate allelopathic effect of *Cannabis sativa* L. extract on activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in leaves of treated plants of *Sorghum halepense* L. Extract was obtained by classical extraction process and applied concentrations were 100%, 50%, 25% and 10%, until control variant was not treated. Plant of *S. halepense* L. was treated under field conditions in initial growth stages. After 6h and 24h from treatment leaves of treated plants were sampled. The samples were monitored for activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Results indicate increased activity of monitored enzymes after 6h only in variant treated with the highest concentration of extract. After 24h enzyme activity was significantly lower compared to the untreated sample. An increase in activity of antioxidant enzymes after 6h is a response to stress caused by applied extract of *C. sativa* L., respectively an allelopathic effect was observed.

Keywords: *Cannabis sativa* L., *Sorghum halepense* L., allelopathy, superoxide dismutase (SOD), catalase (CAT)

Introduction

Invasive plant species have been subject of numerous studies. Finding methods of controlling invasive plant species, with good efficiency and minimizing environmental impact, becoming a goal of today's agricultural production. Allelopathy has great potential for finding new biopesticides. Allelopathy is a relationship in which plants release chemical substances by affecting growth of other plants, inhibiting or stimulating it [1]. Allelopathy is very important in weed control since it does not involve the use of synthetic compounds [2]. Numerous growth inhibitors have been identified in plants that are responsible for allelopathic properties of certain plant species and provide potential for their use in the development of bioherbicides [3]. Some of plant species with proven allelopahic properties are: *Ambrosia artemisiifolia* L., *Avena* sp., *Amaranthus retroflexus* L., *Cyperus esculents* L., *Chenopodium album* L., *Helianthus tuberosus* L., *Rumex crispus* L., *Xantium strumarium* L. [4]. McPartland [5] indicates a repellent and pesticidal properties of *C. sativa* L. Allelopathic effect of extract is highly dependent on applied concentration and length of exposure of treated plant [6]. Barnes & Putnam [7] point to fact that lower concentrations of applied extract may also have higher allelopathic activity in sterile soils as opposed to their activity in non-sterile soils. Allelochemicals in certain plant species can cause oxidative stress, wich is manifested as activation of an antioxidant mechanism [8]. Plants with their enzymatic activity respond to adverse environmental influences [9]. Since enzymes such as superoxide dismutase (SOD) and catalase (CAT) are very important in process of protecting cells from reactive oxygen

species, their activity can be used as an indicator of plant oxidative stress [10]. Aim of this study was to investigate effect of *C. sativa* L. extract on activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in treated *S. halepense* L. plants.

Material and method

Cannabis sativa L. plant material used is of Helena variety, collected at ripening stage in Bački Petrovac during 2018. Dry plant material was crushed and 400g was added to 4l of distilled water. Classical extraction for 24h at 25^{o}C gave extract used, wich was filtered through filter paper. Field sprayer treated field with *Sorghum halepense* L. in initial growth stages. After 6h and 24h from treatment, leaves of treated plants were sampled for biochemical analyzes. Each 2g of fresh leaves sampled was homogenized in 10ml of phosphate buffer (0.1M, pH 7.0). Samples were then centrifuged for 20min at 10000 x g (Boeco, Germany) and filtered. Samples thus obtained were used for further biochemical analyzes. Activity of antioxide dismutase (SOD) and catalase (CAT) was examined. Readings were performed using a Thermo Scientific Evolution 220 (USA) UV/VIS spectrophotometer. Activity of SOD was determined based on photochemical reduction of nitroblutetrazolium (NBT) in which formazan was produced. A sample of 10µL was added to a 1.2ml solution (50mM phosphate buffer (ph 7.8), 13mM L-methionine, 75µM NBT, 0.1mM EDTA) and then 600 µL riboflavin was added. Prepared solution was stirred and placed in front of light source for 20min. The SOD activity unit is amount of enzyme that inhibits NBT reduction by 50% at 25oC and 560nm. CAT activity was determined based on a decrease in H_2O_2 absorption in presence of enzyme extract at 240nm. Unit of catalase activity is amount of enzyme that causes degradation of 1µmol H_2O_2 over a period of 1min at 25^{o}C, expressed per miligram of protein. An enzyme extract obtained from leaves of *S. halepense* L. of 40µL was added to a 2ml solution consisting of 50mM potassium phosphate buffer (pH 7.0) and 10mM H_2O_2. Obtained values of monitored parameters are shown as mean values with standard error. Values were processed by one-way analysis of variance (ANOVA) and comparison test used was Duncan's test in Statistica for Windows. Values indicated by same letters are of same statistical significance at confidence level $P<0.05$.

Results and discussion

Applied concentrations of *Cannabis sativa* L. extract indicate a change in activity of monitored enzymes superoxide dismutase (SOD) and catalase (CAT) relative to control. After 6h of plants treatment with extract only concentration of 100% gave higher activity while other applied concentrations decreased activity of SOD. After 24h of treatment, decreased SOD activity was observed in treated samples except variant with application of a minimum extract concentration of 10%. Obtained values of activity of superoxide dismutase (SOD) enzyme with standard error and statistical significance are shown in Table 1. After 6h of treatment, CAT values increased significantly relative to control. Concentration of 100% gave the highest activity. Unlike 24h post-treatment period where treated samples indicate significantly less CAT activity than control variant. Obtained values of catalase enzyme (CAT) activity with standard error and statistical significance are shown in Table 1.

Table 1. Superoxide dismutase (SOD) and catalase (CAT) activities in leaves of *Sorghum halepense* L. after 6h and 24h after treatment with *Cannabis sativa* L. extract

Enzyme	Concentration	100%	50%	25%	10%	0%
SOD	Activity after 6h	782,61±8,78^{bcd}	650,72±26,52^{ae}	660,14±1,92^{a}	686,95±27,18^{a}	720,29±24,22^{b}
	Activiti after 24h	688,40±16,85^{bde}	634,06±15,34^{acde}	725,36±12,32^{bd}	784,78±8,76^{abc}	761,59±14,97^{ab}
CAT	Activiti after 6h	102,66±36,39^{bcde}	26,75±14,69^{a}	19,93±7,36^{a}	32,42±12,84^{a}	20,55±3,09a
	Activiti after 24h	27,07±9,58^{d}	15,15±6,13^{d}	39,77±5,98	9,43±3,87^{d}	72,75±25,93^{abc}

*values with same letter are at same level of significance (p<0,05)

Allelopathic property of *C. sativa* L. is also proven by Mahmoodzadeh et al [11], Akhter et al. [12], Rueda-Ayala et al. [13] using bioassays, examining effect of applied extract on germination and initial growth of *Lactuca sativa* L. plants.

Conclusion

Our results indicate an increased activity of monitored SOD and CAT enzymes in samples of treated plants *Sorghum halepense* L. only after 6h at the highest concentration of *Cannabis sativa* L. extract. After 24h, there was a descrease in activity of SOD and CAT enzymes in samples treated with extract used. Changes in enzyme activity have been reported to indicate stress in treated plants. Plants treated with the highest concentration of extract exhibit the greatest stress after 6h from time of treatment.

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