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# Advancement in enzyme activities in *Vigna radiata* by allelopathic applications of xanthone extracts of some *Swertia* species collected from Southwest zone of Maharashtra

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**ABSTRACT**

*Swertia species are the valuable medicinal plants. These are the biological sources of useful xanthones, triterpenoids, flavonoids, steroids, sugars and alkaloids. Naturally occurring xanthones are now a day emerged as an important class of organic compounds due to their remarkable pharmacological and other biological applications. In the present study, the allelopathic effect of xanthone extract of three Swertia species was studies with reference to catalase, peroxidase and polyphenol oxidase activity under two different conditions i.e. under lab condition and culture condition. Treatment of xanthone extract was administered with three concentrations, 0.05%, 0.1% and 0.5%. Advancement in the studied enzyme activity was investigated in the culture condition. The activities of Catalase and peroxidase were observed to be enhanced in the experimental set of lab condition but polyphenol activities were decreased.*

**Key words:** Allelopathy, *Swertia* species, Xanthones.

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## MATERIALS AND METHODS

**Plant material-** The authenticated samples were collected from three different localities; *Swertia densifolia* from Kas- Satara Dist., *Swertia lawii* from Panhala- Kolhapur Dist. and *Swertia minor* from Sinhagad - Pune Dist. Voucher specimens have been deposited in Blatter Herbarium, Mumbai India.

**Extraction of crude xanthone-**
Fresh and mature leaves of *Swertia species* were collected randomly, cleaned and shade dried. The dried leaves were powdered and macerated in dichloromethane / methanol (1/1, v/v) for 48 hrs. filtered and the filtrate was concentrated using Rotary evaporator to obtain crude extract. The part of this extract was reextracted with ethyl acetate following Bogne et al., [1] and stored in the fridge for further use.

**Administration of *Swertia* xanthone treatment-**
The allelopathic effect of *Swertia* xanthone extract was investigated by administrating the treatment under two different experimental conditions in *Vigna radiata* leaves.

**Under lab condition-**
The seeds of *Vigna radiata* were treated with xanthone extract of three *Swertia* species in a group of 50 seeds. Three xanthone concentrations 0.05%, 0.1% and 0.5% of the respective *Swertia* species were tried for the treatment. The treated seeds were then taken to test their germination using blotting paper method. Seeds from each treatment were placed on petri dish of 9.0 cm diameter containing water soaked blotting paper for germination. Each treatment was replicated thrice. Leaves of the plants were processed for the evaluation of enzyme activities.

**Under culture condition-**
In another set of experiment, the seeds of *Vigna radiata* were grown in MS media. The media was supplemented with studied concentrations of *Swertia* xanthone extracted from three species. MS medium without xanthone extract was used to grow seeds as a control. The leaves of seven days plant were processed to estimate enzyme activities.

**Enzyme activity study-**
Leaf material obtained from the seeds grown in both the conditions were used for enzyme assay. 1 gm. leaves were weighed accurately and homogenized with 10 ml 0.1 M phosphate buffer (pH 7.0). Centrifuged at 17,000 rpm. for 15 min. The clear supernatant was taken as the enzyme source.

**Catalase activity –**
Catalase (CAT) activity was determined according to Chance and Maehly [2]. 5 ml of the assay mixture comprised, 3 ml phosphate buffer (pH 6.8), 1ml of $H_2O_2$ and 1ml of the twice diluted enzyme extract. After incubation at $25^0$C for 1 min., the reaction was stopped by adding 10 ml of 2% (v/v) $H_2SO_4$ and the residual $H_2O_2$ was titrated against 0.01N $KMnO_4$ until a faint purple colour persisted at least 15 sec. A control was run at the same time in which the enzyme activity was stopped at "zero" time. Activity of enzyme is expressed as mg $H_2O_2$ broken down $min^{-1}$ $g^{-1}$fresh weight.

**Peroxidase activity-**
The method of Maehly and Chance, [3] was followed for the assay of enzyme peroxidase. Enzyme assay mixture was prepared by adding 2 ml 0.1M phosphate buffer (pH 7.0), 1 ml 20 mM Guaicol and 1ml enzyme. The reaction was initiated by addition of 0.04 ml of 10 mM $H_2O_2$ and the change in optical density due to oxidation of Guaicol were recorded at every 30 sec. up to 2 min. at 470 nm with frequent stirring of reaction mixture. The progressive colour change with time represented enzyme activity.

**Polyphenol oxidase assay-**
The Polyphenol oxidase (PPO) activity was assayed as per the procedure of Haplin and Lee [4]. The reaction mixture consisted 1.5 ml of 0.1M Sodium phosphate buffer (pH 6.5) and 200µl of the enzyme extract. 0.3 ml 0.01M Catechol was added to the reaction mixture to start the reaction. PPO activity was expressed as change in absorbance at 412 nm per minute per gram fresh weight of tissue.

## RESULTS AND DISCUSSION

Results obtained on enzyme activity study are tabulated in Table-1, 2 and illustrated in Figure-1 and 2). Enhanced activity of catalase, peroxidase and polyphenol oxidase was observed for most of the studied concentrations of xanthone extracts. The advanced impact was noticed for catalase and peroxidase but polyphenol oxidase activity lowered under lab conditions compared to control (Table-3, figure- 3). The higher average peroxidase activity (1.11 $mg^{-1}min^{-1}$ $g^{-1}$ f. w.) was recorded in lab condition. The increased activity for catalase, peroxidase and polyphenol oxidase was recorded in culture condition, the treatment revealed comparatively better impact in contrast to lab condition. Catalase (4.11 $mg^{-1}$ $min^{-1}$ $g^{-1}$ f. w.) and polyphenol oxidase (1.07 $mg^{-1}$ $min^{-1}$ $g^{-1}$ f. w.) activity was significantly high in xanthone supplemented MS media condition (against respective control) compared to lab condition.

**Table - 1: Impact of *Swertia* crude xanthone extracts on enzyme activity in *Vigna radiata* grown under lab condition**

| Sr. No. | *Swertia* species | Concentration (%) | Catalase ($mg^{-1}min^{-1}$ $g^{-1}$ f.w.) | Peroxidase ($mg^{-1}min^{-1}$ $g^{-1}$ f.w.) | Polyphenol oxidase ($mg^{-1}min^{-1}$ $g^{-1}$ f.w.) |
|---|---|---|---|---|---|
| 1 | Control | - | 5±0 | 0.47±0 | 0.05±0.16 |
| 2 | *S. densifolia* | 0.05 | 4±0.01 | 0.62±0 | 0.01±0.13 |
| | | 0.1 | 4±0.01 | 0.72±0 | 0.01±0.24 |
| | | 0.5 | 6±0 | 1.17±0.01 | 0±0.17 |
| 3 | *S. lawii* | 0.05 | 3±0.01 | 0.96±0 | 0±0.12 |
| | | 0.1 | 6±0.01 | 1.23±0 | 0.01±0.11 |
| | | 0.5 | 7±0.09 | 1.5±0.07 | 0.09±0.11 |
| 4 | *S. minor* | 0.05 | 3±0.01 | 1.24±0 | 0.1±0.12 |
| | | 0.1 | 6±0 | 1.47±0 | 0.01±0.12 |
| | | 0.5 | 8±0.4 | 2.07±0.03 | 0.12±0 |

**Table – 2: Impact of crude xanthone extracts of *Swertia* species on enzyme activity in *Vigna radiata* grown in xanthone supplemented MS media**

| Sr. No. | *Swertia* species | Xanthone Concentrations (in %) | Catalase ($min^{-1}$ $g^{-1}$ f. w.) | Peroxidase ($min^{-1}$ $g^{-1}$ f. w.) | Polyphenol oxidase ($min^{-1}$ $g^{-1}$ f. w.) |
|---|---|---|---|---|---|
| 1 | Control | - | 2±0 | 0.01±0.01 | 0.38±0.01 |
| 2 | *S. densifolia* | 0.05 | 2±0 | 0.23±0.01 | 1.32±0 |
| | | 0.1 | 1±0 | 0.08±0.01 | 0.53±0.05 |
| | | 0.5 | 1±0 | 0.09±0.01 | 0.41±0 |
| 3 | *S. lawii* | 0.05 | 4±0 | 0.19±0 | 0.37±0 |
| | | 0.1 | 4±0 | 0.15±0 | 0.47±0.01 |
| | | 0.5 | 6±0 | 0.16±0.01 | 0.45±0.01 |
| 4 | *S. minor* | 0.05 | 5±0 | 0.3±0 | 3.43±0.01 |
| | | 0.1 | 9±0 | 0.4±0.01 | 0.58±0.02 |
| | | 0.5 | 5±0 | 1.14±0.01 | 2.35±0 |

**Fig.- 1 Impact of *Swertia* crude xanthone extracts on enzyme activity in *Vigna radiata* grown under lab condition**

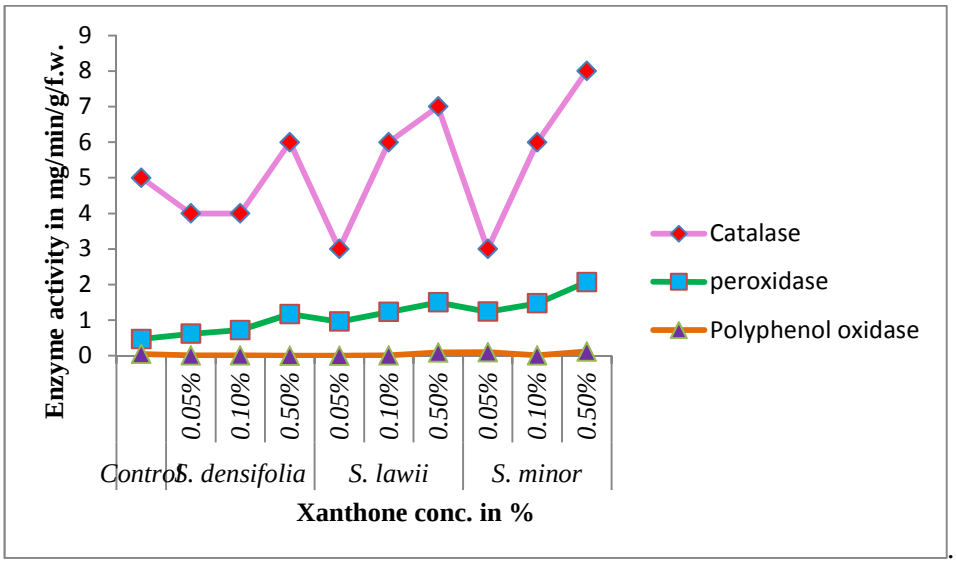



**Fig. – 2 Impact of crude xanthone extracts of *Swertia* species on enzyme activity in *Vigna radiata* grown in xanthone supplemented MS media**

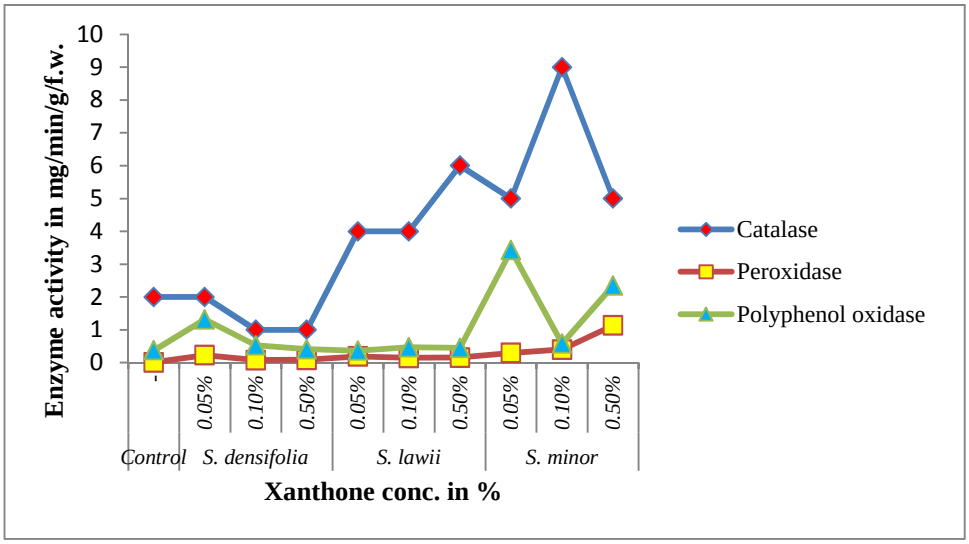
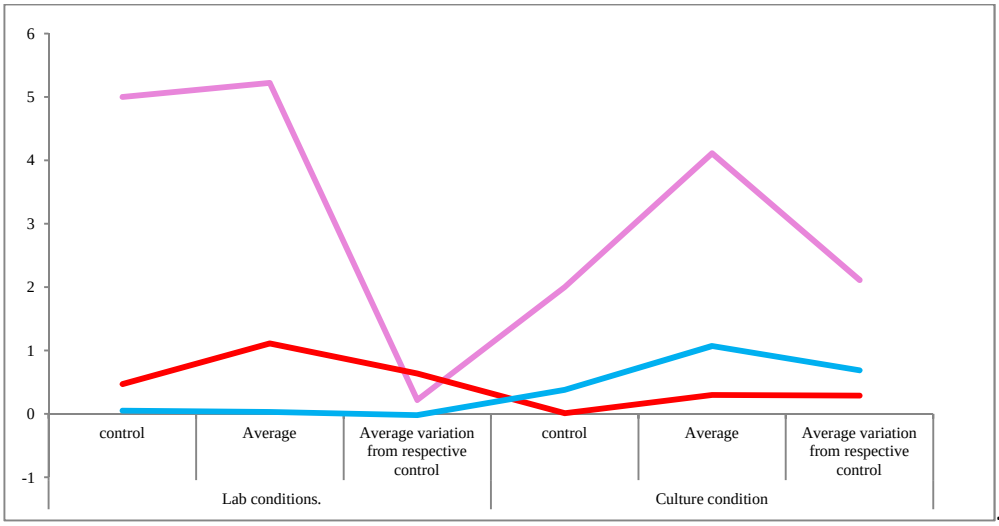


**Table - 3 : Comparative analysis of allelopathic effect of *Swertia* xanthone extracts on *Vigna radiata* in lab condition and media supplemented with xanthone extract**

| Sr. No. | Enzyme activity | Under Lab conditions. | | | Media supplemented with *Swertia* xanthone extracts. | | |
|---|---|---|---|---|---|---|---|
| | | control | Average | Average variation from respective control | control | Average | Average variation from respective control |
| 1 | Catalase ($mg^{-1}min^{-1}g^{-1}$ f. w.) | 5 | 5.22 | 0.22 | 2 | **4.11** | **2.11** |
| 2 | Peroxidase ($mg^{-1}min^{-1}g^{-1}$ f. w.) | 0.47 | **1.11** | **0.64** | 0.01 | 0.30 | 0.29 |
| 3 | Polyphenol oxidase ($mg^{-1}min^{-1}g^{-1}$ f. w.) | 0.05 | 0.03 | -0.02 | 0.38 | **1.07** | **0.69** |

**Fig. – 3 Comparative analysis of enzyme activity studies in *Vigna radiata* leaves obtained from the seeds treated with *Swertia* xanthone extracts under lab conditions and MS media supplemented with xanthone extracts**




Allelopathic potential of leaf extracts and leaf leachates of *Lantana camara* L. on growth of mung bean plant was studied by Maiti et al., [5]. The growth parameters were significantly reduced in seedlings of each concentration. The biochemical changes include: protein, chlorophyll content as well as activity of catalase enzyme. A drastic reduction of proteins and chlorophyll as well as catalase was clearly recorded. An increase in the activity of antioxidant enzymes in response to environmental stresses have been reported in various studies [6]. The catalase activity was higher (9±0 mg/min/g. f. w.) in 0.1% *S. minor* xanthone treated plants grown in culture condition. Rudolph and Stakmann [7] have evaluated the role of catalase in the virulence of pathogen on the host-parasite interaction. The role of catalase as inhibitor of IAA oxidase has been emphasized [8]. In the present work, varied level of CAT activity was observed in xanthone treated seedlings.

Peroxidase is one of the important pathogenesis-related proteins (PR-Proteins). It has dual role in plant defense mechanisms, one as its involvement in reactive oxygen intermediates (ROI) metabolism to generate hydrogen peroxide, and secondly, it is capable of reducing the level of hydrogen peroxide during $H_2O_2$-dependent polymerization of hydroxyl cinnamoyl alcohols (lignin biosynthesis) [9]. Results revealed that activity of Peroxidase (POX) was maximum (2.07±0.03 mg/min/g. f. w.) in 0.5% *S. minor* concentration. Raghvendra et al., [10] suggested increased activity of polyphenol oxidase (PPO), peroxidase (POX), and phenylalanine ammonialyase (PAL) in plants treated with various biotic and abiotic resistance inducers. Increase in oxidizing enzymes particularly polyphenol oxidase (PPO) and peroxidase (POX) has tremendous impact on host physiology and predominantly genes responsible for the resistant pearl millet cultivars [11]. Maximum polyphenol oxidase (PPO) activity was noted in 0.5% S. *minor* xanthone treated seedlings (2.35±0 mg/min/g. f. w.). Grant [12] suggested that pesticides are organic compounds and presence of such compound in a cell might exert some fundamental effect in enzyme production or enzyme function such as induction, repression or feedback inhibition is the possible explanation for the decreasing of mitotic index which might be resulting in complete inhibition of the germination of seeds. Shankar et al. [13] analyzed allelochemicals effect of *Gmelina arborea* on *Vigna mungo* and *Vigna radiata*. The extract inhibited the proteolytic enzyme important for seed germination. The extract inhibited the germination, seedling growth and total protein content of both the test crops.

Oxidative enzymes are considered to be involved in disease resistance in plants; furthermore, a number of studies have indicated that exposure to allelochemicals, which generated superoxide anion radicals, resulted in an increase of peroxidase levels [14]. Hence, the increase of peroxidase activity suggests that peroxidases can contribute to plant defence against potentially toxic compounds [15].

## CONCLUSION

Enhancements in the enzyme activities viz. catalase, peroxidase and polyphenol oxidase in both the condition (except for polyphenol oxidase in lab condition) were evident in the present analysis. Comparative analysis between the lab condition and culture condition showed maximum enzyme activity in culture condition. *S. minor* xanthone treated seedlings have enhancing effect on enzymes when compared to other two *Swertia* species i. e. *S. densifolia* and *S. minor*. These findings would greatly facilitate formulation of agroforestry systems with higher yields by avoiding harmful allelopathic interactions and through exploitation of beneficial effects of particular system. There is a scope in developing resistance varieties by treating with biotic resistance inducer.

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