



## Study of Seed Germination and Enzyme Metabolism During Allelopathic Interaction of *Trianthema portulacastrum L.* and fenugreek seeds

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### Abstract

In the fields, it is commonly observed that fenugreek plants are not growing to their maturity in the vicinity of *Trianthema portulacastrum L.* In this context, present study was conducted in laboratory to observe allelopathic interaction between *Trianthema* and fenugreek. Aqueous extracts of seed, stem and leaf of *Trianthema* were made. Treatment of these aqueous extracts was given to fenugreek seeds. Germination study was performed by using petriplates. All the experiments were performed in triplicates. Pre-treatment of aqueous extracts of seed, stem and leaf of *Trianthema* had caused decline in germination percentage, fresh weight, radical and coleoptile length of fenugreek seeds when compared to untreated seeds. At the same time, mean germination time was found to be more in pre-treated seeds compared to control seeds. After 24 hrs of treatment, enzymatic study was performed. Enzymes from antioxidant metabolism such as peroxidase, ascorbate peroxidase, superoxide dismutase and activity of DPPH was reduced in treated seeds than control seeds. Activity of catalase was increased after treatment of leachates. All the data is graphically presented with the standard deviation. Exogenous treatment of aqueous extracts of *Trianthema* has caused variation in saponin content of fenugreek seeds. This observation was recorded by using finger prints of HPTLC. Finger prints showed that content of saponin was also declined in treated seeds as compared to control. All these observations indicate the strong negative influence of *Trianthema* on growth and metabolism of fenugreek.



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### Introduction


*Trianthema portulacastrum L.* is a herbaceous plant, growing annually. This plant commonly found

in moist area, which spreads on ground not more than 4-6 ft.<sup>6</sup> In India, it is commonly grown as a wild vegetable. This edible plant is vital source of fibres,

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proteins, riboflavin, potassium, sodium and iron.<sup>13</sup> It is also considered as a weed which has many active compounds such as alkaloid, flavonoids, tannins etc. These compounds are also called as allelochemicals, which may stimulate or suppress the growth of neighbouring plants.<sup>26</sup> Study of these allelochemicals and its effects on interaction of plants is called as allelopathy. This is now upcoming branch of biology which involves study of allelochemicals and its influence on plant distribution, building of plant biodiversity and its conservation, evolution of mix crop system in nature and community formation.<sup>33</sup> By exuding these allelochemicals and sharing same ecological conditions such as moisture, nutrients, light, space and air these weeds pose the growth and development of neighbouring plants.<sup>34</sup> Depend upon the type of weed species, its causative effect on seed germination and seedling growth is varied.<sup>8</sup> Plant parts of weed are also equally potential to exert their impression on other plants.<sup>4,1</sup> *Trianthema* is a leafy vegetable with the active wild components. Many workers observed noteworthy observations during allelopathic interaction of this plant. Yield of many economically important plants like cotton, maize, wheat, sorghum, sunflower and mungbean is suppressed due to presence of *Trianthema*.<sup>2,25</sup>

A method of intercropping is followed during cultivation of fenugreek and *Trianthema*. A huge area in Vasai (Maharashtra, India) is under this mix cultivation. But in field, this notorious plant had caused noticeable damage to the fenugreek yield. Considering this on field observations, present study

is conducted. During this study, allelopathic effect of *Trianthema* on fenugreek seed germination, seedling growth and on some enzymes of antioxidant metabolism is evaluated.

**Material and Methods**

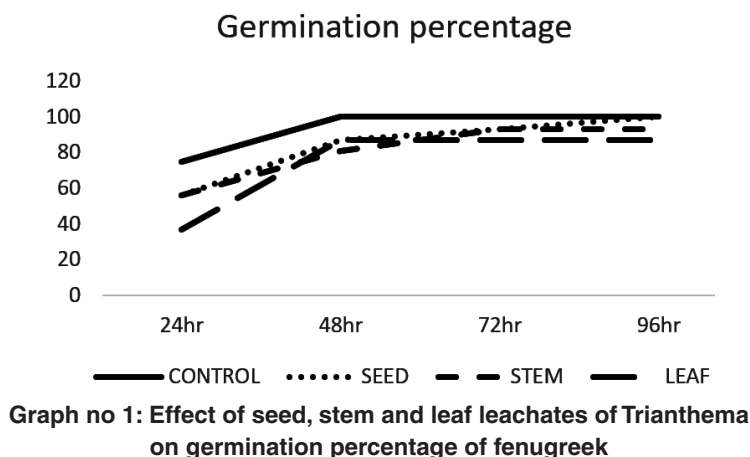
Fresh and matured plants of *Trianthema* were collected from local field of Vasai. Plant parts (seeds, stems and leaves) were separated and sundried for 15 days. Leachates of seed, stem and leaf of *Trianthema* were prepared by following ratio 1/15 (w/v). These leachates were prepared in distilled water and kept for 24 hr. Afterwards, these extracts were filtered through Whatman filter paper No.1. Authorised fenugreek seeds were procured from the market. According to ratio 1/15 (w/v) fenugreek seeds were soaked for 24 hr in seed, stem and leaf leachates of *Trianthema*.

For germination studies, pretreated seeds of fenugreek were kept in three sets of petriplates for 96 hr. Ten millilitre distilled water was poured in each petriplate. Germination count was taken for each day and radical, coleoptile length was measured at 96 hr.

Mean germination time and germination index (GI) were calculated by following the method of<sup>5</sup>

$$\text{Mean germination time} = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds, which were germinated on day D, while D is the number of days, counted from the beginning of germination.



**Graph no 1: Effect of seed, stem and leaf leachates of *Trianthema* on germination percentage of fenugreek**

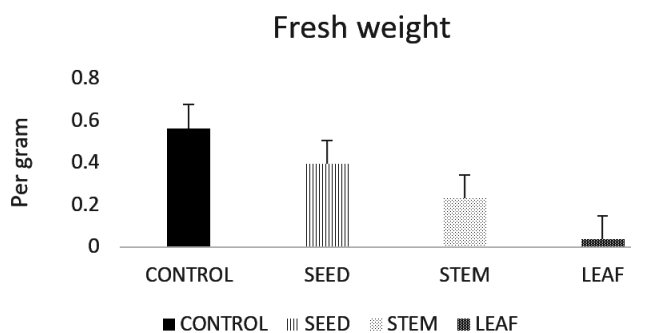
Method<sup>9</sup> was employed for studying enzyme peroxidase. Activity of catalase was determined by following method of<sup>17</sup> as described by.<sup>27</sup> Method of<sup>29</sup> was adopted for determining activity of ascorbate peroxidase. Enzyme super oxide dismutase was determined by using method.<sup>11</sup> Soluble proteins were estimated by the method.<sup>16</sup> Activity of DPPH was estimated using the method described by.<sup>28</sup> Content of saponin was studied using HPTLC finger printing with slight modification in method.<sup>3</sup>

**Result and Discussion**

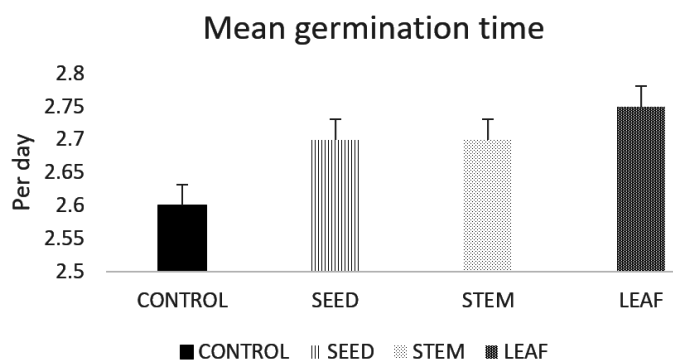
Changes in plants at morphological, biochemical, physiological and molecular level occurred due to environmental conditions. Allelopathy influences other neighbouring plants to change their growth pattern and metabolism. Such changes are also reported in present study. Germination percentage (Graph 1), fresh weight (Graph 2) and mean germination time of fenugreek seeds (Graph 3) is affected after treatment with leachates of

*Trianthema*. More significant reduction in above parameters was noticed in case of leaf leachate treated fenugreek seeds. Seeds of rice were showing similar germination and fresh weight pattern when pre- treated with root, shoot, leaf, seed and whole plant extract of plant *Trianthema*.<sup>14</sup> Many secondary metabolites such as phenols, flavonoids are present in this plant which are responsible for germination retardation. Activity of protease is vital for seed to hydrolyse the proteins, which crucial for imbibition and for water uptake. Soluble phenolic compounds are largely present in leaf leachate of *Trianthema*, which may be responsible for disturbances in seed protease activity. This ultimately leads in more decrease in seed germination of fenugreek seeds pre-treated with leaf leachate compared to seed and stem leachate.

Leachates of *Trianthema* had caused reduction in coleoptile and radical length of fenugreek seeds (Graph 4 and 5).<sup>10</sup> reported decline in germination



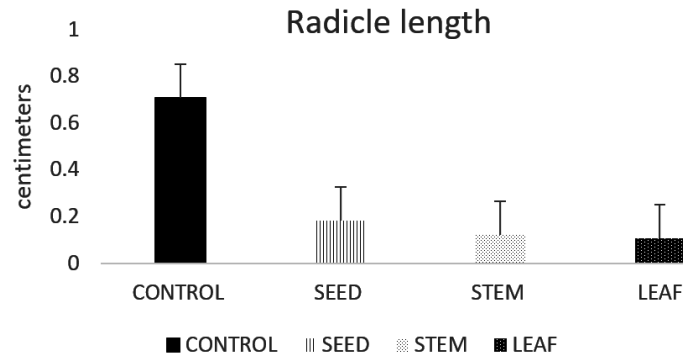
**Graph no. 2: Effect of seed, stem and leaf leachates of *Trianthema* on fresh weight of fenugreek seeds**



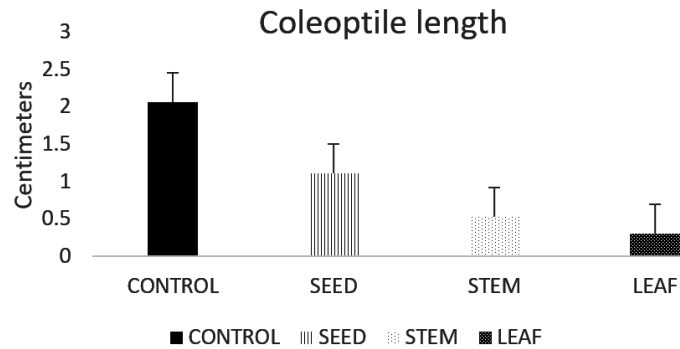
**Graph no 3: Effect of seed, stem, leaf leachates of *Trianthema* on mean germination time of fenugreek**

percentage, plumule and radical length of rice and cowpea, when treated with increasing concentration of *Acacia auriculiformis* leaf leachates. Allelopathic compounds present in water soluble form are

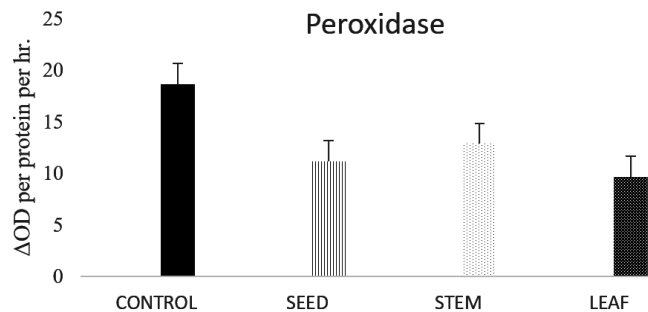
reported to reduce the activity of GA and IAA.<sup>32</sup> This inhibition in hormonal activity results in decline in cell division and reduction in growth of a plant.



**Graph no. 4: Effect of seed, stem and leaf leachates of Trianthema on radicle growth of fenugreek seeds**



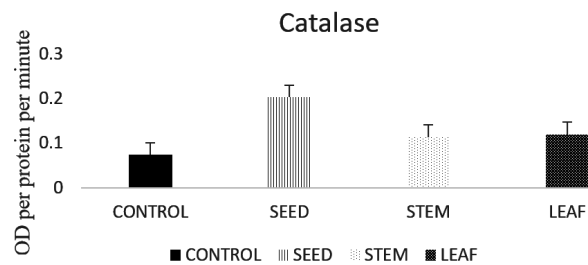
**Graph no. 5: Effect of seed, stem and leaf leachates of Trianthema on coleoptiles growth of fenugreek seeds**



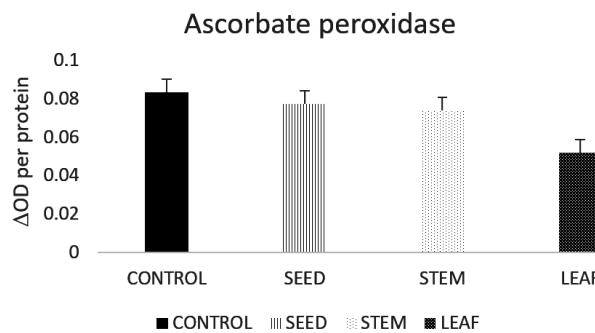
**Graph no. 6: Effect of seed, stem and leaf leachates of Trianthema on enzyme peroxidase of fenugreek**

Stressful environmental situations are responsible for limiting the agricultural productivity, as plants are unable to achieve their maximum genetic strength.<sup>18,7</sup> Sensitive cell organelles of plants react quickly to such environmental changes.<sup>30</sup> Stressful conditions caused increase in ROS in plants. These enhanced ROS are scavenged through many enzymatic and non-enzymatic antioxidants. In present study, activity

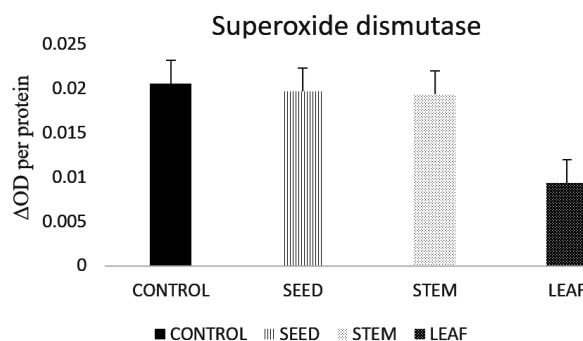
of antioxidant enzyme, peroxidase is decreased in pre-treated fenugreek seeds compared to control (Graph 6). Similar reports are mentioned<sup>24</sup> in *Phalaris* after treatment with extract of *Eucalyptus* leaf. Allelopathic effect of *Trianthema* had caused enhancement in catalase activity of fenugreek seeds (Graph 7). Such kind of enhancement in activity was noticed<sup>19</sup> in Black gram seeds after treatment with



**Graph no. 7: Effect of seed, stem and leaf leachates of Trianthem on enzyme catalase of fenugreek seeds**



**Graph no. 8: Effect of seed, stem and leaf leachates of Trianthema on enzyme ascorbate peroxidase of fenugreek seeds**



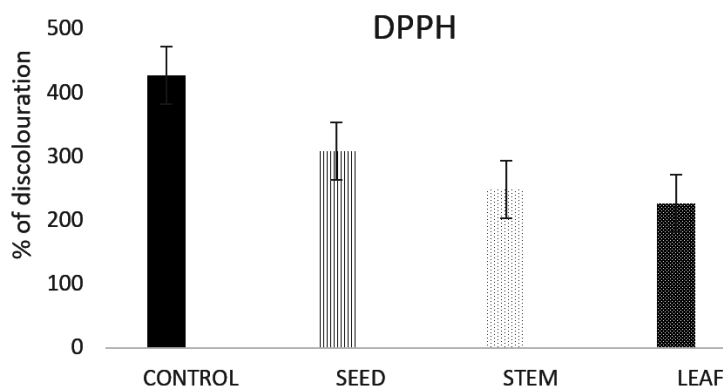
**Graph no. 9: Effect of seed, stem and leaf leachates of Trianthema on enzyme superoxide dismutase of fenugreek seeds**

lower concentration of leaf extract of *Tectona grandis*. In case of fenugreek seeds, as other enzymes are unable to show their antioxidant scavenging activity,

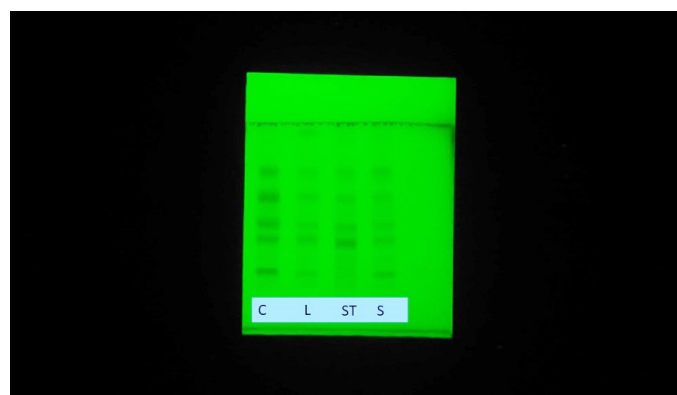
accumulation of H<sub>2</sub>O<sub>2</sub> may be caused in the cell. This harmful compound is efficiently catalysed to H<sub>2</sub>O in peroxisome by increasing the catalase activity in fenugreek. Seed, stem and leaf extract of *Trianthema* had caused decline in enzymatic activity of ascorbate peroxidase in fenugreek seeds (Graph 8). Leaf extract of *Trianthema* had major effect than seed and stem extract. Activity of ascorbate peroxidase in germinating wheat seeds was also hampered by treatment with dried shoot extract of *Achillea santolina* L.<sup>20</sup> In this relation,<sup>15</sup> noticed that a decline in activity of enzyme catalase and enhancement in APX activity in leaves of cucumber seedlings when prone to stress. Similarly, to compensate an increased activity of catalase in fenugreek seeds after allelopathic stress, activity of

**Table no. 1: Allelopathic influence of leaves, stem and seeds of *Trianthema* on saponin content of fenugreek seeds**

Control	Leaves	Stem	Seeds
+++	+	-	++
+++	++	++++	++
++++	+	++	+
++++	+	++	++
++++	+	+	++



**Graph no. 10: Effect of seed, stem and leaf leachates of *Trianthema* on DPPH activity of fenugreek seeds**



**Plate no. 1: Allelopathic influence of leaves, stem and seeds of *Trianthema* on saponin content of fenugreek seeds**

ascorbate peroxidase is decreased. Allelochemicals present in *Trianthema* especially in leaves has caused decrease in activity of enzyme superoxide dismutase (Graph 9). According to,<sup>21</sup> extravagant amount of reactive oxygen species generated may cause reduction in activity of peroxidase and superoxide dismutase. Activity of DPPH in present study showed decrease in pre- treated seeds of fenugreek as compared to control seeds (Graph 10). Chilling stress in rice had caused increase in DPPH activity in tolerant radicles of rice compared to sensitive radicles of rice seedling.<sup>12</sup> This indicates that fenugreek seeds are sensitive to allelopathic stress applied by the *Trianthema*. As noticed on the fields of Vasai, fenugreek seeds are unable to grow and share the same field with *Trianthema*. Laboratory experiments prove the same. Leaves of *Trianthema* possessed more allelopathic compounds than other plant parts.

One of the adaptive strategies of plants to cope up with abiotic stress is to synthesise secondary metabolites such as saponins. Saponin content of seeds of fenugreek was found to be reduced due to allelopathic stress of *Trianthema* leaves, stem and seeds (Plate no.1 and Table no.1). Most noticeable decline in saponin was observed due to extract of *Trianthema* leaves. Similarly,<sup>31</sup> saponin content in roots, leaves, stems, bulbs, flowers and fruit of *Panax ginseng* was negatively affected due abiotic stresses. Combined effect of heavy metals such as

Cu and Cd had caused stressful effect on medicinal plant *Gynura procumbens* (Lour.) Merr. Saponin, Phenolic and flavonoid contents of this medicinal plant were reduced after treating with these metals.<sup>22</sup> According to,<sup>23</sup> phenolic compounds such as caffeic acid, ferulic acid, M- coumeric acid, P- coumeric acid, syringic acid, vanillic acid; and caffeic acid, gallic acid, 4-Hydroxy-3- Methoxybenzoic acid, P-coumeric acid, syringic acid present in *Trianthema* which are responsible for negative allelopathic effect on plants.

### Conclusion

Present study showed that germination ability and seedling growth was badly affected in fenugreek due to treatment of *Trianthema*. Antioxidant enzymes such as peroxidase, ascorbate peroxidase, superoxide dismutase and total antioxidant such as DPPH was also reduced after treatment of *Trianthema*. Leaf lechates of *Trianthema* were found to be more hazardous than other plant parts. Most vital content of fenugreek i.e. saponin was also affected due to treatment of *Trianthema*. Similar kind of observations were noted in fields of Vasai (Dist. Palghar).

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### Conflict of Interest

Authors declare no conflict of interest.

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