Aqueous extract of *Cleistanthus collinus* induces activity of peroxidase and catalase in the seedlings of cucumber and chilli

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We studied the effect of aqueous extracts of *Azadirachta indica*, *Calotropis procera*, *Cleistanthus collinus*, *Dalbergia sissoo*, *Datura inoxia* and *Lantana camara* on induction of peroxidase (POD) and catalase (CAT) in cucumber and chilli seedlings. The extract of *C. collinus*, exhibited a profound effect by inducing maximum activity of POD and CAT in both the crop species. Therefore, we fractionated the extract of *C. collinus* over silica gel column using 90%, 45% and 0% aqueous methanol. The fraction eluted by 90% methanol (fraction 1) showed bioactivity by inducing POD and CAT activity. Also, the extract of *C. collinus* and fraction 1 modulated the isozyme profile of both the enzymes. In addition, they also increased H₂O₂ content in the seedlings, suggesting the triggering of defence mechanism in the seedlings. The findings of the present study suggest the possibility of developing an organic crop protection agent using *C. collinus*.

**Keywords:** Bioactives, crop protection, induced resistance, isozymes, plant extract.

**Protection** of crops from pests and pathogens is an effective way to boost their yield. However, the use of synthetic chemicals results in environmental pollution. Therefore, deployment of biological agents, which trigger the intrinsic defence mechanism of the plants and prevent disease attack on crops, is recommended. Such agents include plant growth-promoting rhizobia (PGPRs) and plant extracts, which are capable of eliciting defence responses in the plants, thereby conditioning them with resistance⁵. Extracts of various plants have been proved to induce systemic acquired resistance (SAR); for example, *Agapanthus africanus*², *Zimmu* (*Allium cepa × Allium sativum*)³, *Allium allicicum*¹, *Adathoda vasica*¹ and *Cinnamomum cassia*¹. Therefore, we aimed to identify a few plants having the ability to trigger defence gene expression in crop plants. This would enable developing a ‘green’ resistance conditioner for the crops. Cucumber and chilli are widely cultivated and consumed the world over. These crops are also susceptible to various diseases⁵,⁶. Hence, induction of defence gene expression by the plant extracts was studied in these two crops. We have recently reported modulation of the activity of chitinases and glucanases by the aqueous extract of *Cleistanthus collinus* in cucumber and chilli⁷. The present study reports the induction of POD and CAT in seedlings of cucumber and chilli by the extracts of wild plants.

**Materials and methods**

**Collection of plants**

Nagpur district, Maharashtra is located in Central India between 20°35′–21°44′N and 78°15′–79°40′E. *Azadirachta indica*, *Calotropis procera*, *Cleistanthus collinus*, *Dalbergia sissoo*, *Datura inoxia* and *Lantana camara* are common all over the region in protected and unprotected forests as well as in areas having human settlements. The aerial parts of these plants were collected from February to May and washed with tap water, shade-dried and pulverized. The powder was stored in airtight plastic pouches under cool and dry conditions until further use. The seedlings of cucumber and chilli were elicited with aqueous extract of these plants.

**Elicitation of seedlings with plant extract and fractionated extract of C. collinus**

The seedlings of cucumber (*Cucumis sativus* L. var. Mohini 186-51585) and chilli (*Capsicum annuum* L. var. Pusa Jwala 172-25624) were elicited with aqueous extract of the plants. The extract was prepared essentially as described earlier¹. The seedlings of cucumber and chilli were raised from the seeds gifted by Ankur Seeds Pvt Ltd, Nagpur, as described by Kadoo and Badere⁷,⁸. The etiolated seedlings of cucumber and chilli were aseptically sprayed with plant extract or fractions of the extract of *C. collinus* and fixed in liquid nitrogen. The seedlings were fixed at 0, 48, 72 and 96 h after elicitation with the plant extract to assay the POD and CAT activity. Similarly, on elicitation with fractions of *C. collinus*...
extract, the seedlings were fixed at 0 and 72 h after elicitation to assay the enzyme activity and develop the isozyme profile of POD and CAT.

Elicited seedlings were fixed in liquid nitrogen between 45 and 75 h after elicitation at an interval of 5 h to study the effect of elicitation with \textit{C. collinus} extract and its fraction 1 on hydrogen peroxide (H$_2$O$_2$) content. The seedlings sprayed with sterile distilled water served as control.

\textit{Fractionation of the extract of C. collinus}

The extract of \textit{C. collinus} was fractionated by sequential elution with 90\% methanol, 45\% methanol and distilled over silica gel column, strictly following the procedure described earlier\cite{7}.

\textit{Enzyme assay}

The activity of POD and CAT was determined in cotyledonary leaves, hypocotyl and root of the seedling, as described by Sadasivam and Manickam\cite{9}.

\textbf{POD assay:} One gram of frozen tissue was crushed with 3 ml of extraction buffer (0.1 M phosphate buffer, pH 7.0) in a prechilled mortar and pestle kept in an ice bucket. The homogenate was centrifuged at 10,000 rpm for 15 min at 5°C and the supernatant used as enzyme source. The 4 ml of the test mixture consisted of extraction buffer, 0.1 ml of crude enzyme extract, 1 μmol of guaiacol and 0.369 μmol of hydrogen peroxide. The contents were mixed and immediately introduced in the UV-Vis spectrophotometer. Absorbance was recorded at 436 nm against a blank prepared by mixing all the ingredients of the test mixture, except H$_2$O$_2$. First, the absorbance was allowed to increase by 0.05 and then the time(s) needed to increase the absorbance by 0.1 was noted. POD activity was calculated according to the following formula

\[
\text{POD activity (units/mg of protein)} = \frac{3.18 \times 0.1 \times p}{6.39 \times \Delta t \times 0.1}
\]

where \(p\) is the amount of protein (mg) present in the enzyme extract and \(\Delta t\) is the time (s) taken to increase the absorbance by 0.1.

\textbf{CAT assay:} One gram of frozen tissue was extracted with 20 ml of extraction buffer (0.0067 M phosphate buffer, pH 7.0) and centrifuging at 10,000 rpm for 10 min. The supernatant was used as crude enzyme extract. The 4 ml of assay mixture consisted of 42.18 μmol of H$_2$O$_2$ in phosphate buffer (0.067 M, pH 7.0), to which 10 μl of crude enzyme extract was added. Absorbance of the assay mixture was read against phosphate buffer (0.067 M, pH 7.0) at 240 nm. The time(s) needed (\(\Delta t\)) for the absorbance to decrease from 0.45 to 0.40 was recorded. CAT activity was determined according to the following formula

\[
\text{CAT activity (units/mg of protein)} = \frac{\Delta t \times 10}{0.01 \times p}
\]

where \(p\) is the amount of protein (mg) in the crude enzyme extract.

The protein content in crude enzyme extract was determined according to Bradford’s method\cite{10}.

\textit{Determination of H$_2$O$_2$ content in the elicited seedlings}

To determine the H$_2$O$_2$ content, 250 mg of tissue was extracted with 5 ml of 0.1\% (w/v) trichloroacetic acid, 10 ml of 1 M potassium iodide and 2 ml of phosphate buffer (10 mM, pH 7.0) in a chilled mortar and pestle over an ice bath. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. Later, absorbance of the supernatant was measured at 390 nm against blank\cite{11}. The amount of H$_2$O$_2$ present in the extract was then determined from the standard curve and expressed as μmol g$^{-1}$ of tissue. The whole procedure was carried out under diffused light.

\textit{Isozyme analysis}

The isoforms of POD were analysed in the crude enzyme extract prepared by homogenizing 1 g of tissue with 2 ml of phosphate buffer (0.1 M, pH 7.0), and centrifuging at 10,000 rpm for 15 min at 4°C. The supernatant was used as enzyme source. Enough volume of extract containing 25 μg of protein was mixed with one-third volume of sample buffer (10% glycerol containing bromophenol blue) and loaded over 7.5% native gel in case of cucumber and 5% native gel in case of chilli. After electrophoresis, the gels were removed and rinsed with phosphate buffer (0.1 M, pH 5.6) for 30 min and immersed in 0.25% guaiacol in the same buffer and finally incubated in 0.15% H$_2$O$_2$ until clear bands developed\cite{12}.

The CAT isoforms were developed from the crude enzyme extract prepared by homogenizing 1 g of tissue with 2 ml phosphate buffer (0.1 M, pH 7) and centrifuging it at 10,000 rpm for 15 min at 4°C. The volume of extract containing 25 μg of protein was mixed with one-third volume of sample buffer and loaded over 7.5% native gel. After electrophoresis, the gels were first incubated in 0.01% H$_2$O$_2$ for 10 min and then in a mixture containing 25 ml each of 2% ferric chloride and 2% potassium ferri-cyanide solution until yellow bands appeared over a dark green background\cite{13}. 

\[\text{RESEARCH ARTICLES}\]
Statistical analysis

Mean, standard error and Student’s t value were computed using MS-Excel and XL-STAT.

Results

Our previous study had reported induction of POD and CAT in the seedlings of cucumber and chilli by biotic and abiotic elicitors. Here we undertook time-course study to determine the time needed by the enzyme to reach its peak activity after elicitation. We found that POD and CAT attained peak activity after 72 h of elicitation. Therefore, in the present study we determined the activity of enzymes at 0, 48, 72 and 96 h after elicitation with the plant extracts. The aqueous extracts of aerial parts of A. indica, C. procera, C. collinus, D. sissoo, D. inoxia and L. camara were sprayed at 2%, 6%, 10%, 16% and 20% concentration to elicit the seedlings. The aqueous extracts of aerial parts of A. indica, C. procera, C. collinus, D. sissoo, D. inoxia and L. camara were sprayed at 2%, 6%, 10%, 16% and 20% concentration to elicit the seedlings. The effect varied according to the crop species, plant extract, concentration of the extract and seedling part. Irrespective of crop species, seedling part and plant extract, POD and CAT achieved peak activity after 72 h of elicitation (Figure 1). The extract of C. collinus induced significant activity of both enzymes in all parts of the seedlings of cucumber and chilli. The extracts of other plants, however, were sporadically active in enhancing enzyme activity (data not shown). Notably, 20% extract of all the plants increased the activity of CAT in chilli (Table 1). In general, 20% extract was the most effective in increasing the activity of enzymes, followed by 16% concentration. In contrast, the lower concentrations, i.e. 2%–10% could not enhance the activity of POD and CAT (data not shown). Since 20% concentration was the most effective and peak activity was reached 72 h after elicitation, we compared the activity of POD and CAT induced by the 20% extract of all the plants after 72 h of elicitation. The results showed that the extract of C. collinus was markedly active in increasing enzyme activity followed by C. procera (Table 1). A differential response of cucumber seedlings in inducing enzyme activity was obvious towards the plant extracts. While the extract of C. procera increased the activity of POD to a maximum; C. collinus induced the activity of CAT to a maximum in cucumber seedlings (Table 1). In contrast, the extract of C. collinus induced maximum activity of POD in chilli seedlings (Table 1). However, the cotyledonary leaves, hypocotyl and roots of chilli seedlings responded differentially to plant extracts in inducing CAT activity. The extracts of C. collinus, L. camara and A. indica induced maximum activity of CAT in cotyledonary leaves, hypocotyl and roots of chilli seedling (Table 1).

The extract of C. collinus, was the most effective in inducing enzyme activity in cucumber and chilli seedlings. Therefore, in the next step we fractionated the extract of C. collinus over the silica gel column. We sequentially eluted the extract of C. collinus with 90% methanol (fraction 1), 45% methanol (fraction 2) and distilled water.
An increase in H2O2 content was recorded (Figure 4). The cotyledonary leaves of cucumber where about 17% after elicitation (Figures 4 and 5). Such a trend was prominent in the cotyledonary leaves and roots of cucumber seedlings after 72 h of elicitation. The other two fractions, could not induce POD and CAT activity (Figures 2 and 3).

Proving that elicitation of seedlings with fraction 1 induces activity of POD and CAT in seedlings prompted us to examine the effects of elicitation with fraction 1 in detail. The first aspect we studied was the effect of elicitation on the H2O2 content in seedling parts. H2O2 content in the seedlings after elicitation varied according to the crop species and seedling part. H2O2 content in the seedlings gradually increased till 70 h before subsiding at 75 h after elicitation (Figures 4 and 5). Such a trend was prominent in the cotyledonary leaves of cucumber where about 17% increase in H2O2 content was recorded (Figure 4 a). Unlike cotyledonary leaves, H2O2 content in hypocotyl and roots of cucumber seedlings showed slight improvement after 70 h of elicitation. On the other hand, H2O2 content in chilli seedlings gradually increased after elicitation in cotyledonary leaves and roots till 70 h after elicitation. In contrast, H2O2 content in hypocotyl of chilli was almost unaffected till 60 h after elicitation. However, a sudden upsurge in H2O2 content was recorded till 70 h after elicitation (Figure 5).

Since reactions catalysed by POD and CAT involve H2O2, we compared the peak activity of the enzymes with maximum H2O2 content recorded in the study in seedling parts (Figure 6). The comparison revealed the expected correlation between H2O2 content and enzyme activity. The seedling parts where the activity of POD was higher also had higher H2O2 content thus showing positive correlation between them (Figure 6 a). In contrast, negative correlation between CAT activity and H2O2 content in the seedlings of cucumber and chilli was apparent (Figure 6 b).

The second aspect about the effect of fraction 1 in seedlings of cucumber and chilli was the elucidation of qualitative change in the induction of POD and CAT. We developed the zymogram of each enzyme to achieve this objective. A remarkable change in the induction of isoforms of POD and CAT was evident from a study of isozyme profiles of the enzymes in seedling parts (Figures 7–10). The seedling parts of cucumber showed two heavy and a moderate-sized band corresponding to POD. However, on challenge with plant extract and fraction 1, the isozyme profile was altered in the cotyledonary leaves and hypocotyl. The plant extract and fraction 1 induced an extra band each of heavy, moderate and lightweight in the cotyledonary leaves of cucumber (Figure 7 a). In the same manner a single heavy isoform of POD was induced in the hypocotyl of cucumber by plant extract and fraction 1. In addition, elicitation with the plant extract and fraction 1 induced several moderate-sized isoforms of POD in the hypocotyl (Figure 7 b).

Unlike cucumber, tissue-specific isoforms of POD were present in the seedling parts of chilli. The cotyledonary leaves of chilli had two heavy isoforms and one isoform each of moderate and lightweight (Figure 7 d). In contrast, the hypocotyl of chilli had three heavy bands of POD isoforms and one isoform of moderate size (Figure 7 e) and the roots of chilli had two heavy bands and one band each of moderate and lightweight (Figure 7 f). Eliciting the seedling with plant extract and fraction 1 induced one moderate weight band in cotyledonary leaves of chilli. Also, elicitation with fraction 1 induced a heavy band of POD in cotyledonary leaves (Figure 7 d). Similarly, elicitation also induced extra isoforms of POD in hypocotyl of chilli. While plant extract induced one lightweight band in hypocotyl, elicitation with fraction 1 induced two lightweight bands of POD (Figure 7 e). Elicitation with plant extract and fraction 1 brought about the most significant changes in the zymogram of POD in the roots of chilli seedlings. First, the intensity of moderate

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Table 1. Peak activities of enzymes in the seedlings elicited with 20% plant extract

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Peroxidases (units/mg of protein)</th>
<th>Catalases (units/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cotyledonary leaves</td>
<td>Hypocotyl</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>Calotropis procera</td>
<td>0.58</td>
<td>0.18</td>
</tr>
<tr>
<td>Cleistanthus collinus</td>
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<td>0.11</td>
</tr>
<tr>
<td>Dalbergia sissoo</td>
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<td>0.06</td>
</tr>
<tr>
<td>Datura inoxia</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>0.08</td>
<td>0.04</td>
</tr>
</tbody>
</table>

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Figure 2. Induction of peroxidases by the aqueous extract of *C. collinus* and its fraction 1 in the seedlings of cucumber and chilli. *a*-c, Cucumber: *(a)* cotyledonary leaves, *(b)* hypocotyl and *(c)* roots. *d*-f, Chilli: *(d)* Cotyledonary leaves, *(e)* hypocotyl and *(f)* roots. *Significant at 5% level of confidence according to Student’s *t*-test.

and lightweight bands increased prominently and second, elicitation induced an extra lightweight band in the roots of chilli (Figure 7*f*).

Similar to POD, obvious changes in the zymogram of CAT were noticed on elicitation with plant extract and fraction 1. However, unlike POD, the new isoforms of CAT were seldom induced. The cotyledonary leaves of cucumber showed the presence of one band each of heavy, moderate and lightweight. Notably, the moderate weight band occupied larger area than the other two bands. Elicitation of cucumber seedlings with plant extract further increased the area occupied by the moderate weight band. Strikingly, elicitation of cucumber seedling with fraction 1 induced an extra equally large moderate weight band in the cotyledonary leaves (Figure 8*a*). The hypocotyl and roots of cucumber showed a heavy weight band with a prominent moderate weight band in control. However, eliciting the seedlings with
Figure 4. Hydrogen peroxide (H$_2$O$_2$) content in cucumber seedlings elicited with the aqueous extract of _C. collinus_ and its fraction 1. (a) Cotyledonary leaves; (b) hypocotyl and (c) roots.

Figure 5. H$_2$O$_2$ content in chilli seedlings elicited with the aqueous extract of _C. collinus_ and its fraction 1. (a) Cotyledonary leaves, (b) hypocotyl and (c) roots.

Figure 6. Correlation between enzyme activity and H$_2$O$_2$ content in the seedlings elicited with the extract of _C. collinus_.

Discussion

Use of plant extract as a natural plant defence inducer is worthwhile to prevent environmental and toxicological problems. Such products would have multiple benefits like eco-friendly nature, yield-boosting action, narrow target range, specific mode of action and low persistence in the environment$^{15}$. Plants synthesize secondary
metabolites like phenols, phenolic acids, quinones, flavones, flavonoids, flavanols, tannins, coumarins, etc. These secondary metabolites are part of the defence mechanism of plants against pests and pathogens. Besides, they are a rich source of bioactive compounds which can be used as novel crop-protecting agents.

The extracts of Zizyphus jujuba and Ipomoea carnea protect rice plants from Rhizoctonia solani by inducing defence enzymes like PAL, chitinases, β-1,3 glucanase, peroxidase and accumulating phenolics at a higher level. Similarly, induction of antioxidant enzymes and resistance against Fusarium wilt in tomato seedlings on elicitation with aqueous extracts of neem and willow have also been reported. The extracts of Cymbopogon citrus and Ocimum sanctum have also been reported. In the same line, increase in POD activity up to six days in the fruits of banana dipped in leaf extracts of Solanum torvum, Zimmu and A. aliiaceum has been proved. In conformation with earlier reports our study also revealed the induction of POD and CAT by extracts of A. indica, C. procera, C. collinus, D. sissoo, D. innoxia and L. camara. However, the ability to induce POD and CAT activity varied among the extracts. The qualitative and quantitative chemical analysis of the powder of plants selected for the study revealed the diversity of phytochemicals in them. C. collinus showed the presence of terpenoids, tannins, flavonoids, saponins, anthraquinones, coumarins and lignans, suggesting its richness in phytochemicals. C. collinus tested positive for lignans and negative for alkaloids. Similarly, cardiac glycosides were present only in D. sissoo, while tannins and coumarins were present in all the plant samples (data not shown). The quantitative analysis revealed that C. collinus had phenolic content much higher than the other plant samples. Similarly, D. sissoo and L. camara had higher content of carbohydrates and soluble proteins (data not shown). Therefore, the ability of plant extracts to induce POD and CAT activity in the seedlings of cucumber and chilli might be because of chemicals present in them. However, differential activity of the extracts with respect to crop species and seedling parts was also evident. This might be because of (i) absence of the chemicals that can induce POD and CAT genes; (ii) presence of chemicals that can induce POD and CAT genes at suboptimal concentration, or (iii) differential distribution of cell-surface receptors over the seedlings.

A difference of opinion exists among researchers about the role of POD in plant defence. While some argue for a
direct role of POD in defence reaction of plant resistance\textsuperscript{21}, others regard POD activity as a biochemical marker which may not necessarily be a part of the resistance mechanism\textsuperscript{22,23}. Metabolism continuously produces reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical and H\textsubscript{2}O\textsubscript{2} as by-products\textsuperscript{24}. It has been proved that ROS, H\textsubscript{2}O\textsubscript{2} in particular, are the components of early defence responses in plants, which include direct antimicrobial action, lignin formation, phytoalexin production and triggering SAR\textsuperscript{25}. However, simultaneously ROS also harm the plant cells. When produced in excess, ROS cause oxidative damage of pigments, proteins, nucleic acids and lipids. Therefore, for normal plant growth, ROS should be maintained at sub-lethal level. To ensure this, plants have developed protective mechanisms against oxidative stress. These mechanisms use enzymatic and non-enzymatic molecules to scavenge the excess ROS. Enzymatic scavenging of ROS includes multiple antioxidant enzyme systems. For example, superoxide dismutase reacts with the superoxide radical to produce H\textsubscript{2}O\textsubscript{2}, which is scavenged by POD and CAT. The non-enzymatic constituents are tocopherol, ascorbate and reduced glutathione which remove or neutralize ROS\textsuperscript{26}. In the present study, elicitation of seedlings with extract of C. collinus and its fraction 1 led to increase in H\textsubscript{2}O\textsubscript{2} content. Therefore, the consequent increase in activity of POD and CAT because of elicitation probably is to scavenge the excess H\textsubscript{2}O\textsubscript{2} from the cells. Since the extract of C. collinus had a broad spectrum of activity, we decided to study its fractions eluted over silica gel column. Therefore, the extract of C. collinus was fractioned sequentially with 90%, 45% and 0% aqueous methanol. Only fraction 1 (eluted with 90% methanol) showed bioactivity by inducing POD and CAT activity. Moreover, the bioactivity of fraction 1 was mostly higher than that of the plant extract. As stated earlier, bioactivity of plant extracts is due to the presence of certain chemicals in them, which can interact with the receptors over the
Figure 10. Gels showing catalase isoforms. a–c, Cucumber: (a) Cotyledonary leaves; (b) hypocotyl and (c) roots. d–f, Chilli: (d) Cotyledonary leaves, (e) hypocotyl and (f) roots.

cells and elicit response. Display of bioactivity exclusively by fraction 1 suggests that all the bioactive compounds were eluted in it, while the remaining two fractions contained none of the bioactive principles or they were present at suboptimal levels.

Considering the bioactivity of fraction 1, we studied its effect in more detail. For this, we determined H$_2$O$_2$ accumulation by the seedlings and modulation of isozyme profile of the seedlings on elicitation with extract of $C$. collinus and fraction 1. Although the difference between the effect of plant extract and fraction 1 on H$_2$O$_2$ concentration in seedling was inconspicuous, the isozyme profiles induced by these two elicitors were noticeably different, at least occasionally. Moreover, the isoforms of POD and CAT were also tissue-specific. We have earlier reported the quantitative characterization of the extract of $C$. collinus and fraction 1 (ref. 7). Fraction 1 is richer compared with the plant extract in terms of carbohydrate and flavonoid content. In contrast, plant extract is richer in total protein and tannin content than fraction 1. We have also reported the TLC profile of alkaloids, sesquiterpene lactone, terpenoids, lignans, tannins, coumarins, flavonoids, phenylpropanoids, anthraquinones and sapo

in the extract of $C$. collinus and fraction 1. While the plant extract profile was mostly similar with that of fraction 1; certain derivatives of phytochemicals were unique to either of them7. Therefore, probably the quantitative and qualitative differences in phytochemicals of the $C$. collinus extract and its fraction 1 are responsible for induction of different isoforms of POD and CAT in the seedlings of cucumber and chilli.

H$_2$O$_2$ content in the seedlings elicited with $C$. collinus extract and its fraction 1 correlated with the POD and CAT activity. Elicitation or infection causes oxidative burst inside the cells27, which signals activation of defence genes28. The findings of the present study suggest activation of defence response in seedlings because of elicitation by extract of $C$. collinus and fraction 1. Our
earlier study on induction of the activity of β-1,3 glucanase and chitinases by the aqueous extract of *C. collinus* supports this assumption.

Further, in this study increase in H$_2$O$_2$ content in seedlings was observed after challenging with the extract of *C. collinus* and its fraction 1. H$_2$O$_2$ content increased after elicitation and then subsided after 70 h, when the POD and CAT activity was at its peak. The initial increase in H$_2$O$_2$ content after elicitation is in agreement with the earlier findings, where such increase was observed on application of chemical agents in drought-stressed rice plants$^{29}$. In the present study, the H$_2$O$_2$ content was inversely proportional to the CAT activity for which H$_2$O$_2$ induces SAR is supported by studies involving the application of chemical agents in drought-stressed rice and then subsided after 70 h, when the POD activity and CAT due to infection and application of pod extracts. Induction of POD isoforms in tobacco on infection by tobacco mosaic virus and its positive correlation with induced resistance have been established$^{30}$. Similarly, variation in the expression of POD isoforms in tomato, cowpea and soybean by the application of *Trichoderma asperellum*$^{30}$, carrot root extract$^{40}$ and garlic extract$^{41}$ respectively, has been reported.

**Conclusion**

The findings of present study establish the induction of POD and CAT by the aqueous extract of *C. collinus*. It further supports our earlier proposition that the bioactivity of *C. collinus* could be exploited for crop protection in the field$^{7}$. However, a comprehensive field study is needed in this regard.


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