Oxidative Response of Groundnut (*Arachis hypogaea*) Plants to Salicylic Acid, Neem Oil Formulation and *Acalypha fruticosa* Leaf Extract

1Abdul Rashid War, 1Shanmugavel Lingathurai, 1Michael Gabriel Paulraj, 2Mohd Yousf War and 1Savarimuthu Ignacimuthu
1Entomology Research Institute, Loyola College, Chennai, Tamilnadu, 600034, India
2Degree College Sopore, JandK, 193 201, India

Corresponding Author: S. Ignacimuthu, Entomology Research Institute, Loyola College, Chennai, Tamilnadu, 600034, India Tel: 91-44-28178348 Fax: 91-44-28175566

ABSTRACT

Plants are always under threat due to various biotic and abiotic stresses. They respond to such threats by an efficient antioxidative system. A series of experiments were carried out to study the effect of exogenous application of Salicylic Acid (SA), *Acalypha fruticosa* chloroform leaf extract and Neem Oil formulation (NO) on the induction of antioxidative enzymes such as Peroxidase (POD), Polyphenol Oxidase (PPO) and on total phenols, hydrogen peroxide ($H_2O_2$) and protein contents in groundnut plants. Twenty day-old plants were sprayed with SA (0.014 and 0.028%), *A. fruticosa* extract (1.0%) and NO (0.2, 0.5 and 1.0%). Water treated plants were maintained as control. A considerable increase in the oxidative enzyme activities, total phenols and protein contents was observed at 24, 48, 72 and 96 h after treatment. A quick response was shown by plants to *A. fruticosa* (1.0%) application. Extract of *A. fruticosa* (1.0%) induced maximum enzyme activities (13.1 IU g$^{-1}$ FW POD and 0.48 IU g$^{-1}$ FW PPO, respectively, at 96 h after treatment). Moreover, total phenols, $H_2O_2$ and protein contents were also high in *A. fruticosa* treated plants followed by those treated with NO (1%). These findings showed that *A. fruticosa* extract and neem oil influenced the metabolic system in plants and induced the oxidative response that could defend plants against a variety of stresses.

Key words: Antioxidative enzymes, peroxidase, polyphenol oxidase, plant defense, induced resistance

INTRODUCTION

Plants face numerous stresses in their life and respond to them through several physical and chemical characteristics (Omid, 2010). The oxidative state of plants plays a pivotal role in plant defense against such stresses. Induction of various antioxidative enzymes and other defensive compounds is a common phenomenon in plants in response to different biotic and abiotic stresses (Omid, 2010). This response occurs both in the plant organs originally attacked (local response) and also in non-attacked organs (systemic response) (Metraux et al., 2002). Various defensive compounds accumulate in plant tissues in response to microbial, fungal and herbivore attack or when sprayed with chemicals including Salicylic Acid (SA) (Corlach et al., 1996; Gulser et al., 2005; Ansari and Misra, 2007). Most of the biotic and abiotic stresses lead to
increased production of Reactive Oxygen Species (ROS) such as superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH) (Noreen and Ashraf, 2009; Heidari, 2009; He et al., 2011; Gorji et al., 2011). Plants protect themselves from cytotoxic effects of these ROS with the help of antioxidant enzymes such as Peroxidase (POD), Polyphenol Oxidase (PPO), Phenylalanine Ammonia Lyase (PAL), Catalase (CAT) and Superoxide Dismutase (SOD) induced in plants in response to the stress (Josef and Jini, 2010; Rani and Jyothsna, 2010; He et al., 2011). Induced oxidative response enables the plants to cope with various kinds of stresses and allows them to be phenotypically plastic. It makes the plant more unpredictable for stress causing agents due to the variations in defense constituents of the plant (War et al., 2011a, b).

SA is a well known naturally occurring signaling molecule that plays a key role in establishing and signaling plant defense responses against various pathogenic infections (Radhakrishnan and Balasubramanian, 2009), insect pests (Peng et al., 2004; Zhao et al., 2009) and abiotic stresses (Ansari and Misra, 2007; Joseph et al., 2010; Syeed et al., 2011; Idrees et al., 2011). Induction of resistance through elevation of oxidative state in plants by exogenous application of SA, acetyl salicylic acid, polyacrylic acid, methyl salicylate, jasmonic acid, jasmonate methyl ester and other chemicals has been well documented by Metraux et al. (2002), Syeed et al. (2011), Idrees et al. (2011) and War et al. (2011a).

Several plant extracts have shown insecticidal activity and have been exploited for insect pest management (Baskar et al., 2009). Plant secondary metabolites play an important role in plant defense against insects and pathogens (Cai et al., 2004), the levels of which are often mediated by antioxidant enzymes such as PAL, PPO, POD and phenolics (Paul and Sharma, 2002; Kagale et al., 2004; Cavalcanti et al., 2006; Rani and Jyothsna, 2010; War et al., 2011a, b). Insecticidal properties of Acalypha fruticosa Forssk (Euphorbiaceae) chloroform extract at 1.0% have been studied earlier; also preliminary phytochemical analysis of chloroform extract of A. fruticosa showed the presence of many secondary metabolites (Lingathurai et al., 2010).

Peroxidases comprise a class of proteins which catalyze the lignin synthesis and therefore, may be directly associated with increased ability of systemically protecting the tissue by lignification when plants are threatened by herbivores, microorganisms and abiotic stresses (Zhao et al., 2009; Islam et al., 2011; Idrees et al., 2011; War et al., 2011a, b). PPOs catalyse the O$_2^-$-dependent oxidation of phenolics to quinones and have been proposed as a component of elaborate plant defense mechanisms against pathogens, insects and wounding (Ramiro et al., 2003; Bhonwong et al., 2009; War et al., 2011a, b). Plant phenolics include several groups of compounds such as simple phenols, phenolic acids, flavonoids, isoflavonoids, tannins and lignins. Many plant phenolic compounds are known to function as precursors to structural polymers such as lignins or serve as signal molecules that defend plants against various stresses (Baker et al., 2005). It has been broadly accepted that H$_2$O$_2$ is involved in a number of signaling cascades in plants (Neill et al., 2002), including the response to biotic (He et al., 2011; War et al., 2011a, b) and abiotic stresses (Chen et al., 1993; Noreen and Ashraf, 2009; Idrees et al., 2011).

Groundnut (Arachis hypogaea L.) is one of the most important oilseed crops throughout the world. Globally groundnut cultivation occupies about 23.4 million ha with an annual production of 34.9 million metric tons (http://www.fao.org/docrep/010j994e/j994e04.htm). A number of biotic and abiotic factors pose a great threat to this crop (War et al., 2011a, b).

To defend themselves against different stresses, plants have developed a wide range of physical and chemical mechanisms that has been well documented (Rani and Jyothsna, 2010;
Idrees et al., 2011; Syeed et al., 2011; War et al., 2011a, b). However, such reports are limited in groundnut. Since groundnut faces a great threat from various biotic and abiotic factors, the present study was carried out to find out whether SA and botanicals alter the activities of antioxidant enzymes, amount of total phenols, hydrogen peroxide and proteins in this crop. Here, we focused on POD and PPO activities, total phenols, \( \text{H}_2\text{O}_2 \) and protein contents, since these are the most studied components implicated in plant defense.

**MATERIALS AND METHODS**

**Chemicals:** The chemicals used in this study were of analytical grade. Salicylic Acid (SA), Tris-HCl, Polyvinyl Pyrrolidone (PVP), EDTA, disodium hydrogen phosphate, sodium dihydrogen phosphate and guaiacol were obtained from HiMedia Lab. Pvt. Ltd., 2-mercaptoethanol from Loba Chemie, Pyrocatechol from Central Drug House, Coomassie brilliant blue-G250 from Sisco Research Lab., Bovine Serum Albumin (BSA), potassium iodide (KI) and Sodium carbonate (\( \text{Na}_2\text{CO}_3 \)) from S.d. Fine Chemicals Ltd. and Gallic acid and Folin-Ciocalteau reagent were obtained from Merck, Mumbai, India.

**Neem oil formulation:** The Neem Oil formulation (NO) was prepared using neem oil (45%), karanj oil (45%), azadirachtin technical (0.05%), karajin technical (0.05%) and emulsifier (DMA-NE) (7.8%). Neem oil, Karkan oil, Azadirachtin and karanj were gifted by Nimbion Organics, Chennai, India. The ingredients were added in a mixer and stirred for 30 min using an electric stirrer.

**Acalypha fruticosa extract:** To prepare *A. fruticosa* extract, fresh leaves were collected from Marudhamalai hills in Coimbatore, Tamil Nadu, India. The species was identified by a taxonomist and a voucher specimen was deposited in the Herbarium at the Entomology Research Institute, Loyola College, Chennai, India. Leaves were dried under shade and powdered by an electric blender. Nearly 300 g of leaf powder was soaked in 1 L chloroform for 72 h and filtered through a Whatman No. 1 filter paper. The extract was condensed under reduced pressure using vacuum evaporator (Equitron EV 11,F. 012).

**Plant material and exogenous application of salicylic acid and *A. fruticosa* extract:** The present study was carried out in 2008-2009 at the Entomology Research Institute, Loyola College, Chennai, India. Groundnut variety JL-24 was used as the model plant in this study. Groundnut plants were grown in pots with mixture of soil, sand and vermicompost (2:1:1) in a net house at Entomology Research Institute, Loyola College, Chennai, India. The plants were watered as needed. Twenty days old groundnut plants were sprayed with various concentrations of SA (0.014 and 0.028%), *A. fruticosa* (1.0%) and NO (0.02, 0.5 and 1.0%). Water treated plants were used as a control. After 24, 48, 72 and 96 h of spray, fully expanded leaves were collected randomly from the treated plants and used for biochemical attributes.

**Enzyme extraction:** Fresh leaves (0.5 g) were frozen in liquid nitrogen and ground in 3 mL of ice cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1% Polyvinylpyrrolidone (PVP) and 0.5 mM EDTA. The homogenate was centrifuged at 14,000 rpm for 25 min and the supernatant used as an enzyme source. All spectrophotometric analyses were carried out on HITACHI UV-2010 spectrophotometer.
Enzyme assays: POD activity was estimated as per the method of Shannon et al. (1966) with a slight modification. The enzyme activity was measured as IU g\(^{-1}\) FW. One unit of POD activity was defined as the change in absorbance by 0.1 units per minute under conditions of assay.

The PPO activity was estimated as per the method of Mayer and Harel (1979) with some modifications. The enzyme activity was measured as IU g\(^{-1}\) FW. One unit of PPO was defined as the change in absorbance by 0.1 unit per minute under conditions of assay.

Hydrogen peroxide content: H\(_2\)O\(_2\) content was estimated by the method of Noreen and Ashraf (2009). H\(_2\)O\(_2\) concentration was determined by using an extinction coefficient of 0.28 \(\mu\)M cm\(^{-1}\) and expressed as \(\mu\)mol g\(^{-1}\) FW.

Phenolic content: Amount of total phenols in treated and control plants were estimated by using the method of Zieslin and Ben-Zaken (1993), with some modifications. Phenolic content was determined from a standard curve prepared with Gallic acid and expressed as \(\mu\)g Gallic acid equivalents g\(^{-1}\) FW (\(\mu\)g GAE g\(^{-1}\) FW).

Protein estimation: Protein content was determined by the method of Bradford (1976) using BSA as standard.

Statistical analysis: Data were subjected to Analysis of Variance (ANOVA) using SPSS (Version 11.1). When the treatment effects were statistically significant (\(p = 0.05\)), the Tukey’s test was used to test the significance of difference between the treatment means.

RESULTS

POD activity: There was a general increase in POD activity of plants in all the treatments as compared to the control plants (Table 1). However, A. fruticosa (1.0%) treatment induced significantly higher activity across the test period (3.8, 6.5, 12.3 and 15.1 IU g\(^{-1}\) FW, respectively, at 24, 48, 72 and 96 h) than the other treatments. At 96 h, plants treated with Neem oil 1% exhibited POD activity (12.2 IU g\(^{-1}\) FW) that was at par with that induced by A. fruticosa (1.0%).

PPO activity: The PPO activity was significantly greater in plants treated with SA and botanicals compared to the untreated control plants (Table 2). Plants sprayed with A. fruticosa (1.0%) showed higher PPO activity at all the time intervals (0.25, 0.35, 0.43 and 0.48 IU g\(^{-1}\) FW, respectively, at 24, 48, 72 and 96 h) as compared to other treatments. Although, progressive elevation in PPO

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
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</thead>
<tbody>
<tr>
<td>SA 0.014%</td>
<td>2.3±0.9</td>
<td>5.3±1.3</td>
<td>6.8±1.6</td>
<td>8.5±1.3</td>
</tr>
<tr>
<td>SA 0.028%</td>
<td>2.2±0.1</td>
<td>5.5±1.1</td>
<td>6.9±1.9</td>
<td>10.3±1.7</td>
</tr>
<tr>
<td>Neem oil 0.2%</td>
<td>3.7±1.0</td>
<td>4.1±1.1</td>
<td>5.5±0.9</td>
<td>6.8±2.0</td>
</tr>
<tr>
<td>Neem oil 0.5%</td>
<td>3.2±0.6</td>
<td>5.2±0.5</td>
<td>5.2±0.6</td>
<td>6.4±1.8</td>
</tr>
<tr>
<td>Neem oil 1%</td>
<td>3.4±0.3</td>
<td>4.6±0.7</td>
<td>7.4±1.1</td>
<td>12.2±2.1</td>
</tr>
<tr>
<td>A. fruticosa (1%)</td>
<td>3.8±0.8</td>
<td>6.5±1.6</td>
<td>12.3±1.1</td>
<td>15.1±1.6</td>
</tr>
<tr>
<td>Control -water</td>
<td>2.1±0.6</td>
<td>3.4±0.1</td>
<td>3.1±0.5</td>
<td>4.2±1.2</td>
</tr>
</tbody>
</table>

Values (Mean±SEM) with similar letter (s) within a column are not significantly different by Tukey’s test (\(p = 0.05\)). FW: Fresh weight of leaf tissue \(n = 10\) for each treatment.
Table 2: Polyphenol oxidase activity (IU g⁻¹ FW) in groundnut plant after the application of salicylic acid and botanicals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA 0.014%</td>
<td>0.14±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23±0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.30±0.002&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SA 0.028%</td>
<td>0.07±0.000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25±0.006&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.27±0.004&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neem oil 0.2%</td>
<td>0.15±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29±0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27±0.004&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.30±0.005&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neem oil 0.5%</td>
<td>0.14±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23±0.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24±0.005&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26±0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neem oil 1%</td>
<td>0.20±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35±0.010&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.38±0.004&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. fruticosa</em> (1%)</td>
<td>0.23±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35±0.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.48±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control - water</td>
<td>0.12±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20±0.008&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.21±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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</table>

Values (Means±SEM) with similar letter(s) within a column are not significantly different by Tukey's test (p = 0.05). FW: Fresh weight of leaf tissue n = 10 for each treatment.

Table 3: Hydrogen peroxide content (μmol g⁻¹ FW) in groundnut plants after application of salicylic acid and botanicals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA 0.014%</td>
<td>14.5±1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.2±3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.5±4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.8±5.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SA 0.028%</td>
<td>15.3±2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.9±2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.4±4.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.2±3.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neem oil 0.2%</td>
<td>15.9±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.1±2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.7±2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.5±3.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neem oil 0.5%</td>
<td>16.3±3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.1±4.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.1±4.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.4±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neem oil 1%</td>
<td>17.3±2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.4±3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.9±1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.5±3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. fruticosa</em> (1%)</td>
<td>24.4±9.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.1±5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.4±3.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.4±6.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control - water</td>
<td>11.0±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.4±2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.0±1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.1±2.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values (Means±SEM) with similar letter(s) within a column are not significantly different by Tukey's test (p = 0.05). FW: Fresh weight of leaf tissue n = 10 for each treatment.

Activity was observed in all the treatments, however, the expression rhythm differed in various treatments and different time intervals.

**Hydrogen peroxide content:** Significantly higher H₂O₂ content was observed in treated plants than the untreated controls (Table 3). Among the treatments, higher accumulusion of H₂O₂ was recorded in plants treated with *A. fruticosa* (1.0%) (24.4, 26.1, 29.4 and 34.4, respectively, at 24, 48, 72 and 96 h) than the other treatments.

**Total phenols:** Total phenol content increased in all treatments as compared to the control plants up to 72 h after treatment (Fig. 1). Significant increase in phenolic content was recorded in *A. fruticosa* (1.0%) treated plants. The elevation in phenolic content was very high at 72 h (142.6 µg GAE g⁻¹ FW) after treatment. Least phenolic content was recorded in control plants (57.4 µg GAE g⁻¹ FW).

**Protein content:** The protein content of the leaves of the plants treated with SA and botanicals was significantly greater (Fig. 2) than the untreated control plants. Protein content of plants treated with chloroform extract of *A. fruticosa* 1.0% was very high (p<0.01) at 72 h followed by those treated with SA 0.014% (p<0.05).
Fig. 1: Total phenols (µg GAE g⁻¹ FW) in groundnut plants after application with salicylic acid and botanicals. *On the bullets at a given time interval shows significance among the treatments at p<0.05. GAE g⁻¹ FW: Gallic acid equivalents per gram fresh weight SA: Salicylic acid; n = 10 for each treatment

Fig. 2: Protein content (mg mL⁻¹) in groundnut plants after application with salicylic acid and botanicals. *On the bullets at a given time interval shows significance among the treatments at p<0.05. SA: Salicylic acid; n = 10 for each treatment

DISCUSSION

Plants have developed a highly sophisticated antioxidative defense system to cope with many biotic and abiotic stresses (Heidari, 2009; Joseph and Jini, 2010). Utilization of plant’s own defense mechanism against stresses is an attractive strategy that enables the plants to thrive well in hostile environment. Role of SA has been well documented in the activation of defense responses against various biotic and abiotic stresses (Fernandes et al., 2003; Ansari and Misra, 2007; Zhao et al., 2009; Idrees et al., 2011). Plant products play an important role as antifungal,
antibacterial and antiviral agents (Paul and Sharma, 2002; Kagale et al., 2004; Cavalcanti et al., 2006). A strong but positive correlation has been found between oxidative state of the host plant and resistance against different stresses (Zhao et al., 2009; He et al., 2011; War et al., 2011a, b). This oxidative state of the host plant is mediated through the production of ROS and their subsequent elimination by antioxidative enzymes. POD is one such enzyme of prime importance in plant defense that eliminates the ROS, besides its other defensive roles (Idrees et al., 2011). The present study showed that SA and botanicals induced the POD activity in groundnut plants. The induction was significantly greater in plants treated with chloroform leaf extract of A. fruticosa than those sprayed with NO and SA. Present results are in accordance with the earlier reports where induction of POD activity was higher in botanical treated plants. For instance, Schneider and Ullrich (1994) reported that cucumber and tobacco plants treated with extract of Reynoutria sachalinensis had increased activities of POD, PPO, chitinase and β-1, 3-glucanase. Kagale et al. (2004) observed an increase in antioxidant enzymes in rice plants sprayed with Datura metel leaf extract. Increase in POD activity has also been observed in the sesame plants treated with Azadiracta indica leaf extract (Guleria and Kumar, 2006). Kazemi et al. (2011) reported induction of POD activity in Kiwifruit in response to SA application. POD is widely distributed in higher plants and protects the plant cells against the destructive influence of H₂O₂ by catalyzing its decomposition (Lin and Kao, 2002; Fernandes et al., 2006). The induction of POD activity in plants occurs in response to numerous biotic and abiotic stimuli, including exposure to pathogens, insects or elicitor preparations, chemical oxidizing agents and mechanical stimuli (Fernandes et al., 2006; Idrees et al., 2011; Syed et al., 2011; War et al., 2011a, b; Verma et al., 2011). The roles that peroxidase can play in cell wall toughening and in the production of toxic secondary metabolites and its simultaneous oxidant and antioxidant properties, make it an important factor in the defense response of plants to a variety of stresses (Fernandes et al., 2006; Han et al., 2009; Idrees et al., 2011).

The present study suggested that the activity of PPO increased throughout the test period. The induction was more in plants sprayed with chloroform leaf extract of A. fruticosa than the other treatments. It has been found that oxidation of chlorogenic acid by PPO in tomato foliage is associated with a reduction in the growth rates of beet armyworm (Spodoptera exigua) larvae on foliage. Also many of the amino acids most susceptible to derivatization by PPO-generated quinines (i.e., amino acids with nucleophilic properties such as cysteine, methionine, lysine and histidine), were limiting for the growth of lepidopteran larvae (Felton et al., 1992). The PPO activity is also associated with resistance against phloem-feeding and leaf-chewing insects (Stout et al., 1998; Ramiro et al., 2006). PPOs have been found to be induced in response to signaling molecules and injuries inflicted by wounding, pathogens or insect pests in various plant species (Cooper et al., 2004; Ramiro et al., 2006; Bhonwong et al., 2009; War et al., 2011a, b). Induction of PPO activity by botanicals might enable the plants to resist the oxidative damage caused by different stresses.

Phenolics accumulated after exogenous application of salicylic acid and botanicals. The increase was high at 72 h of treatment, after which there was a slight decrease in phenolic content in all the treatments. There was more elevation of phenols in plants treated with botanicals as compared to those treated with SA. Among the botanicals, the increase was greater in plants treated with A. fruticosa extract (1.0%), followed by NO treatments. Phenols constitute a structurally diverse and ubiquitous plant compounds that play a variety of roles in plant defense as phytoanticipins, phytoalexins, structural barriers, modulators of pathogenicity and activators of plant defense genes.
(Rani and Jyothsna, 2010; War et al., 2011a, b). Oxidation of phenols by PPO leads to formation of quinones and free radicals that can activate enzymes, which form a part of the metabolic processes acting against pathogens and insects (Appel, 1993; Bhon Wong et al., 2009). Several studies have shown that plant resistance to both abiotic and biotic stresses is mediated by phenolic compounds (Dicko et al., 2005; Sharma et al., 2009; War et al., 2011a, b). Present results agree with the earlier reports where plant extracts induced the phenolic content. For instance, treatment of cucumber with leaf extracts of R. sachalinensis, resulted in accumulation of phenols and protected the plants against Sphaerotheca fuliginea (Daaye et al., 1995). Barley treated with Azadirachta indica leaf extract induced phenolic content and protected the plants against leaf stripe disease (Paul and Sharma, 2002).

The $H_2O_2$ was increased in plants after treatment. The induction was more in plants treated with A. fruticosa (1.0%) as compared to the plants treated with SA and NO. Oxidative state of the host plant which is mediated through the production of ROS, plays an important role in plant defense against various stresses (Laloi et al., 2007). Among the ROS, $H_2O_2$ is a relatively stable, partially reduced form of hydrogen, diffuses freely and thus is an important factor in generation of defense responses in plants (Boka et al., 2007). A close interaction occurs between perception of $H_2O_2$ in response to biotic and abiotic stresses in plant systems (Maffei et al., 2006; Noreen and Ashraf, 2009; Syeed et al., 2011; Idrees et al., 2011). $H_2O_2$ acts through signal transduction pathways which leads to the expression of defense genes (Orozco-Cardenas and Ryan, 1999; Idrees et al., 2011). Accumulation of $H_2O_2$ instigates a cascade of events that trigger physiological and molecular responses in plants to defend plants against biotic and abiotic stresses (Torres et al., 2006; Maffei et al., 2006; War et al., 2011a, b).

Protein content increased in plants treated with SA and botanicals. Although elevation in protein content was more in plants treated with A. fruticosa (1.0%) as compared to SA and NO treated plants, the differences were not statistically significant. The increase in protein content was reflected in increased POD and PPO activities. Paul and Sharma (2002) reported similar results with Azadirachta indica leaf extract treated barley plants against leaf stripe disease. Increase in protein content plays an important role in plant defense (Chen et al., 2009; War et al., 2011a, b).

CONCLUSION

Botanicals can play an important role in inducing host plant resistance against different stresses by acting as signaling molecules through the induction of antioxidant system. Although the exact mechanism of how the enzyme induction occurs could not be interpreted at this juncture, there is a need for in-depth studies to understand the underlying phenomenon.

REFERENCES


