J Plant Dis Prot (2017) 124:163–175
DOI 10.1007/s41348-016-0067-3

ORIGINAL ARTICLE

A potent biocide formulation inducing SAR in plants

Joyeeta Mitra[1] · **P. K. Paul**[2]

Received: 19 July 2016 / Accepted: 15 December 2016 / Published online: 23 December 2016

Abstract Plants extracts possess biocidal activity which can effectively control numerous plant pathogens. In the present study, antimicrobial activity of formulation prepared from aqueous extract of tender core of *Musa acuminata* pseudostem and *Tagetes erecta* leaves against pathogens infecting cucumber, barley, spinach and tomato was studied. The extract induced biochemical defense in the host plants, which was sustained for several weeks. The newly emerged leaves in each test species were also protected against their respective pathogens. The activity of peroxidase and polyphenol oxidase was significantly enhanced by the extract formulation, leading to the enhancement of systemic acquired resistance in the host plants. The source materials are by-products of banana and marigold cultivation, which at present have no commercial utility.

Keywords Biocide · *Musa acuminata* · POX · PPO · SAR · *Tagetes erecta*

Introduction

Plants are attacked by an array of pathogens [1]. However, they possess structural and biochemical, defense mechanisms which need to be activated against the invading pathogens. An array of botanical extracts have been found to have antimicrobial properties and can induce defense responses in plants against numerous pathogens, thereby increasing yield of crops [2, 3].

The ripe banana fruit peel and pulp possesses antifungal and antimicrobial properties [4, 5]. Fagbemi et al. [6] demonstrated the control of clinical isolates of *Stapyhlococcus aureus* ATCC 25921, *Salmonella paratyphi*, *Shigella flexnerii*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Pseudomonas aeruginosa* using crude ethanolic extracts of unripe banana. Ehiowemwenguan et al. [7] reported that aqueous and ethanolic extract of banana peel could significantly reduce the growth of pathogenic bacteria.

Tagetes erecta has been reported to have insecticidal properties. Marigold roots produce compounds like α-terthienyl that are allelopathic to many species of plant-parasitic nematodes [8, 9]. Maradufu et al. [10] and Weaver et al. [11] used compounds extracted from the leaves and flowers of *Tagetes* which were toxic to *Aedes aegypti* (L.) larvae and Mexican Bean Weevils (Coleoptera: Bruchidae), respectively. Cold aqueous extracts (20% w/v) from whole plant, root and stem of *Tagetes erecta* collected at pre- and post-flowering stages could control *Meloidogyne incognita* in *Lycopersicon esculentum* [12]. Şesan et al. [13] reported the efficacy of hydroalcoholic extracts of *Tagetes* in controlling *Botrytus cinerea.*

The present study was conducted on tomato, barley, cucumber and spinach plants. Tomato is the world's most cultivated vegetable crop. In India, it is an important vegetable eaten raw and cooked. However, its yield is severely impaired by several bacterial pathogens causing speck disease. Barley has globally the maximum acreage under cultivation. In India, the crop is gaining importance because of its wide application in brewing industry. Its

✉ P. K. Paul
prabir_kp@rediffmail.com

[1] Credora Life Sciences, Kalyan Nagar, Bangalore, Karnataka 560043, India

[2] Amity Institute of Biotechnology, Amity University Uttar Pradesh, Express Highway, Gautam Budhha Nagar, Noida 201303, India

yield is affected mainly by *Dreschlera graminea*. Cucumber is the important component of salad, as being eaten raw. It also forms an important source material for many Ayurvedic (traditional) medicine formulations. Spinach is economically an important culinary vegetable and is a rich source of iron and vitamin C. It is highly consumed by Indian population which is predominantly vegetarian. Yield of both spinach and cucumber is severely impacted in India by several fungal, bacterial and viral pathogens.

To boost yield of all these crops, farmers use large quantities of pesticides which are not only environmentally hazardous, but are also responsible for several health hazards in humans. Therefore, it is very pertinent to develop suitable biocontrol strategies for controlling these pathogens to increase yield.

In the Asian subcontinent particularly India, banana is an important commercial crop. The leaves of banana have been reported to have antimicrobial properties against several clinical pathogens [14, 15]. Oil extract of *Tagetes* leaves has been studied previously for their biocidal activity [16–18]. In the current study, the biocidal function of water extracts of pseudostem of banana and the leaves of *Tagetes* was studied which, at present, are considered as agricultural wastes. Their antimicrobial properties to combat plant pathogens have not been exploited.

In the present study, efficacy of a suitable formulation from aqueous extract of banana pseudostem and *Tagetes erecta* leaves in controlling *Alternaria alternata, Pseudomonas syringae* pv. *spinacae, Pseudomonas syringae* pv. *tomato* and *Dreschlera graminea,* respectively, on cucumber, Spinach, Tomato and Barley was studied. The study stressed upon the induction of defense enzymes and establishment of systemic acquired resistance (SAR) by the biocide formulation in the host plants. The broad spectrum biocide would be environmentally safe and cost-effective as it would be derived from agricultural waste. The components of the biocontrol agent were selected on the basis of earlier studies wherein their efficacy as a biocide has been demonstrated.

The present study would unravel the understanding of biochemical aspects of biocontrol by these extracts and help in developing a suitable broad spectrum biocide for commercial application.

Materials and methods

Extraction and preparation of plant extract

Pseudostem of banana and leaves of *Tagetes* was collected from the Organic farm of Amity University Uttar Pradesh, NOIDA, India.

The outer layer(s) of tender core of banana pseudostem was peeled off, and the inner soft core (100 g) was homogenized in 100 mL of sterile distilled water under aseptic conditions [19]. The homogenate was filtered through 8-layered muslin cloth and then through 0.22-mm membrane filters.

One hundred grams of *Tagetes* leaves was homogenized in 100 mL of sterile distilled water under aseptic conditions [20]. The homogenate was filtered through 8-layered muslin cloth and then through 0.22-mm membrane filters.

Both filtrates were mixed in 1:1 ratio and designated as extract. 1:10 and 1:100 dilution was prepared from this extract.

Raising of plants

Seeds of *Cucumis sativus*, *Spinacia oleracea, Solanum lycopersicum* and *Hordeum vulgare* were sown in sterile soilrite in plastic trays (35 cm × 25 cm × 6 cm; L × W × H). Plants were raised under aseptic conditions at 25±1 °C, 70% RH and a photoperiod of 12L/D hours. Sterile Hoagland's solution was added weekly to provide the essential nutrients necessary for plant growth.

Eight-week-old tomato and spinach, 7-week-old cucumber and 10-day-old barley plants were used for the study.

Spraying of plants

Each test plant was divided into 12 groups each containing 50 plants and treated as follows:

Group	Treatment
Group 1	Plants treated with sterilized distilled water (control)
Group 2	Plants treated with pathogen*
Group 3	Plants treated with extract only
Group 4	Plants treated with premix of pathogen* and extract
Group 5	Plants treated with premix of pathogen* and 1:10 dilution of extract
Group 6	Plants treated with premix of pathogen* and 1:100 dilution of extract
Group 7	Pathogen* inoculated 24 h prior to treatment with extract
Group 8	Pathogen* inoculated 24 h prior to treatment with 1:10 dilution of extract
Group 9	Pathogen* inoculated 24 h prior to treatment with 1:100 dilution of extract
Group 10	Pathogen* inoculated after 24 h of treatment with extract
Group 11	Pathogen* inoculated after 24 h of treatment with 1:10 dilution of extract
Group 12	Pathogen* inoculated after 24 h of treatment with 1:100 dilution of extract

* Pathogen inoculated

- *Alternaria alternata* on cucumber.
- *Pseudomonas syringae* pv. *spinacae* on spinach.
- *Pseudomonas syringae* pv. *tomato* on tomato.
- *Dreschlera graminea* on barley.

The above-mentioned 12 groups were repeated for all host-pathogen system. Three replicates were taken for each group. Leaf samples were collected in triplicate from all group replicates at 0, 24, 48, 72, 96 h, 2+ weeks and 3+ weeks intervals.

Disease incidence and severity

The disease incidence and severity on tomato and spinach was calculated by observing the plants and their infected leaves after one week of treatment [21].

Percentage disease incidence = (average number of infected leaves per plant × 100)/average total number of leaves per plant.

Disease severity (%) = sum of rating (0–3 scale) × 100/(maximum possible score × no. of leaves observed).

For barley and cucumber, disease incidence was calculated after 1 week of treatment. According to Arabi and Jawhar [22], disease incidence in barley and cucumber was recorded as the proportion of diseased plants divided by the total number of plants sampled. Disease severity was calculated as infected leaf area per plant expressed as a proportion of the total area. Study was performed in sets of triplicate for each plant group and for each treatment.

Analysis of peroxidase (POX) and polyphenol oxidase (PPO)

Three hundred milligrams of frozen leaf tissue was homogenized at 4 °C in ice-cold sodium phosphate buffer (0.1 M, pH 9.0) containing 10 mM β-mercaptoethanol, 1 mM phenyl methyl sulphonyl fluoride, 0.001% Triton X-100, 1 mM ethylene diamine tetraacetic acid and 10% (w/w) polyvinylpyrrolidone. The homogenate was centrifuged at 15000×*g* for 20 min [23]. The supernatant was used as crude extract for estimation of activities of peroxidase and polyphenol oxidase. Three replicates were taken for each group for each plant. Protein concentration was estimated by Bradford Test [24]. Each replicate had equal concentration of protein.

To prepare Bradford reagent, 100 mg of Coomassie Brilliant Blue G-250 was dissolved in 50 ml of 95% ethanol, and subsequently, 100 ml of 85% *o*-phosphoric acid was added. Bovine serum albumin (BSA) standards were prepared in range of 0–100 mg/ml, and their absorbance was recorded at 595 nm on a UV–Vis spectrophotometer (Shimadzu 1650). A standard graph was plotted, and the protein concentrations of the samples were determined from the graph.

The reaction mixture of POX assay consisted of 0.05 mL crude extract, 1.655 mL of distilled water, 0.245 mL of 1 M sodium phosphate buffer (pH 7.0), 0.25 mL of 100 mM guaiacol and 0.05 mL of hydrogen peroxide (100 mM). The reaction mixture for PPO consisted of 0.5 mL of sodium phosphate buffer (1 M, pH 9.0), 1.25 mL of catechol (0.2 M), 0.05 mL of enzyme extract and 0.2 mL of distilled water. The reaction mixture for both POX and PPO was incubated at 25 ± 1°C for 5 min and then was stopped by addition of 0.5 mL of sulfuric acid (10% v/v). The absorbance was recorded at 470 nm/420 nm for POX/PPO, respectively, on a UV–Vis spectrophotometer, Shimadzu 1650 [25]. Reaction mixture without enzyme extract served as blank. The experimentally taken molar extinction coefficient for guaiacol was 26.6 mM^{-1} cm^{-1} (Costa et al. 2005) and 24.9 cm/min for catechol [26]. Enzyme activity was expressed as units gm^{-1} fresh weight min^{-1}. Enzyme activity and the specific enzyme activity were estimated for sample replicates for all groups.

Native basic PAGE and in-gel activity staining

The isozyme pattern of POX was analyzed by in-gel activity staining using guaiacol as substrate [27]. After Native Basic PAGE, the gels were placed in a substrate solution containing 10 mM Guaiacol in 0.1 M sodium phosphate buffer (pH 7.0) at room temperature for 10 min. Drops of H_2O_2 were gradually added to the gel and shaken gently until orange-colored bands appeared. The isoforms were calculated by relative distance (Rf value) of each isozyme band from each zymogram.

The isozyme pattern of PPO was analyzed by in-gel activity staining using catechol as substrate [28]. The gels were equilibrated in a solution composed of 0.1% p-phenylenediamine in 0.1 M sodium phosphate buffer (pH 7.0) at room temperature for approximately 30 min. This was followed by addition of 10 mM catechol in the same buffer. The gels were shaken gently till discrete dark brown bands appeared. The isoforms were distinguished by calculating the relative distance (Rf value) of each isozyme band.

Isozyme patterns of POX and PPO were studied for all sample replicates of all groups. All the samples loaded had equal concentration of protein.

Statistical analysis

Data for disease incidence and severity were statistically analyzed by SPSS software (version 17) for windows (SPSS Inc., Chicago, Illinois, USA) using univariate

Table 1 Percent (%) disease severity on the new leaves emerging one week after the treatment of plants with Banana pseudostem and *Tagetes* leaves aqueous extract

Treatments	Disease severity in plants (%)			
	Tomato	Spinach	Barley	Cucumber
Pathogen	91.44 ± 3.5^{a}	93.70 ± 3.9^{a}	92.28 ± 3.9^{a}	91.16 ± 4.8^{a}
P + E	70.68 ± 5.1^{ab}	69.78 ± 4.9^{abc}	68.54 ± 7.6^{bc}	69.11 ± 2.4^{bcd}
P + E (1:10)	57.85 ± 0.9^{b}	53.24 ± 2.9^{bc}	55.33 ± 0.8^{c}	53.65 ± 3.3^{cd}
P + E (1:100)	79.55 ± 6.9^{ab}	79.63 ± 2.4^{ab}	79.88 ± 8.7^{b}	77.64 ± 4.2^{ab}
P/E	70.91 ± 4.2^{ab}	69.96 ± 2.0^{abc}	72.08 ± 6.5^{bc}	69.70 ± 3.7^{bcd}
P/E (1:10)	59.82 ± 5.5^{b}	58.40 ± 7.3^{bc}	50.34 ± 15.8^{c}	52.35 ± 12.9^{d}
P/E (1:100)	80.01 ± 4.9^{ab}	84.92 ± 4.9^{ab}	81.93 ± 5.4^{ab}	76.43 ± 8.5^{ab}
E/P	74.57 ± 4.8^{ab}	77.55 ± 9.0^{bc}	70.64 ± 3.8^{bc}	68.20 ± 1.3^{abc}
E (1:10)/P	55.03 ± 2.9^{b}	57.07 ± 3.7^{d}	53.51 ± 2.8^{c}	51.39 ± 4.5^{d}
E (1:100)/P	82.72 ± 3.1^{ab}	81.65 ± 5.2^{ab}	79.32 ± 2.6^{ab}	74.92 ± 1.8^{abc}

Pathogen: plants treated with pathogen; *P + E*: Plants treated with premix of pathogen and extract; *P + E* (1:10): Plants treated with premix of pathogen and 1:10 dilution of extract; *P + E* (1:100): Plants treated with premix of pathogen and 1:100 dilution of extract; *P/E*: Pathogen inoculated 24 h prior to treatment with extract; *P/E* (1:10): Pathogen inoculated 24 h prior to treatment with 1:10 dilution of extract; *P/E* (1:100): Pathogen inoculated 24 h prior to treatment with 1:100 dilution of extract; *E/P* (1:1): Pathogen inoculated after 24 h of treatment with extract; *E/P* (1:10): Pathogen inoculated after 24 h of treatment with 1:10 dilution of extract; and *E/P* (1:100): Pathogen inoculated after 24 h of treatment with 1:100 dilution of extract (*E*—1:1 mix of aqueous extract of soft inner core of banana pseudostem and leaves of *Tagetes erecta*). Percentage of disease severity was averaged for three replicates ±standard error. Different letters within each column indicate that they are significantly different from each other. Data were analyzed using ANOVA and treatment means separated with Bonferroni at Tukey's HSD at $\alpha = 0.05$

general linear model procedures and one-way analysis of variance (ANOVA), respectively, followed by post hoc comparisons using Bonferroni.

The data for POX and PPO were statistically analyzed for ANOVA using the general linear model procedure and the least squares means test of the statistical software SAS (version 9.2 developed by SAS institute Inc., Cary, NC, USA). Multiple pairwise comparison tests using least square means were performed for post hoc comparisons after two-way ANOVA with treatment and time as two factors with replications and using Bonferroni procedure.

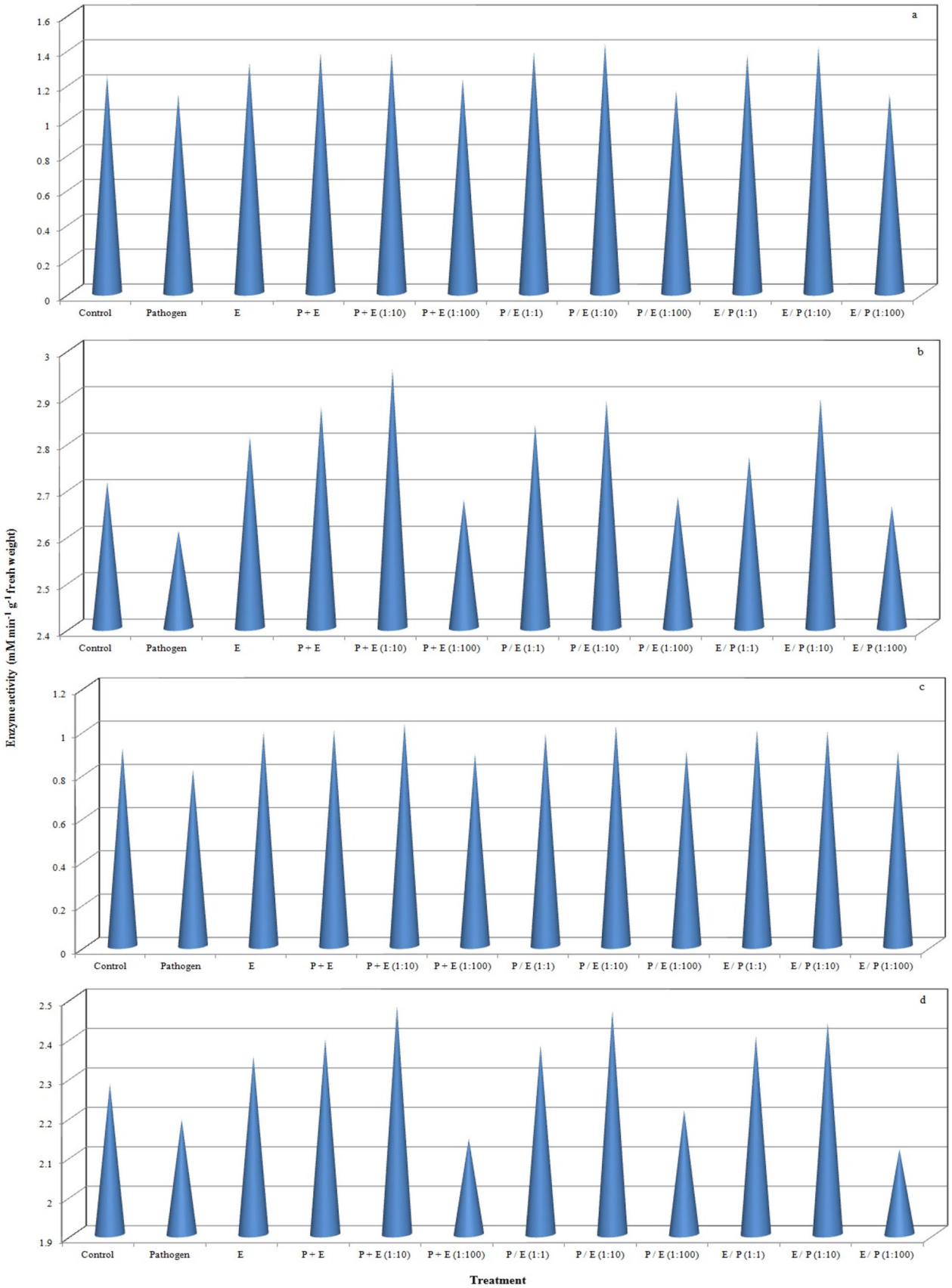
Fig. 1 Peroxidase activity in **a** cucumber plants; **b** spinach plants; **c** tomato plants; and **d** barley plants. ▤: Plants treated with sterilized distilled water (control); ▩: plants treated with pathogen; ⬚: plants treated with extract; ■: plants treated with premix of pathogen and extract; ▣: plants treated with premix of pathogen and 1:10 dilution of extract; ▪: plants treated with premix of pathogen and 1:100 dilution of extract; ▬: pathogen inoculated 24 h prior to treatment with extract; □: pathogen inoculated 24 h prior to treatment with 1:10 dilution of extract; ⬚: pathogen inoculated 24 h prior to treatment with 1:100 dilution of extract; ◪: pathogen inoculated after 24 h of treatment with extract; ▭: pathogen inoculated after 24 h of treatment with 1:10 dilution of extract; and ▥: pathogen inoculated after 24 h of treatment with 1:100 dilution of extract. $*p \leq 0.05$; $**p \leq 0.01$. $^{\Psi}$*Vertical bars* represent standard error

Results

Analysis of results demonstrates that application of aqueous extract mix of tender core (pseudostem) of banana and marigold leaves on the surface of the sterile leaves of host plants affected the disease development on the plants. Substantial reduction in disease symptoms and induction in activity of POX and PPO was observed after extract treatment.

Tomato plants treated with 1:10 dilutions of aqueous extract was most effective ($P \leq 0.05$) in inducing SAR against *Pseudomonas syringae* pv. *tomato*, and disease severity was highly reduced (Table 1) from 94 to 57% as compared to its controls. In Spinach, the incidence of *Pseudomonas syringae* pv *spinacae* was reduced from 93 to 53% ($P \leq 0.05$) on application of 1:10 dilution of extract (Table 1). *D. graminea* on barley could be controlled within 7–8 days of treatment with undiluted extract mix, but the effect was more pronounced when it was diluted 10 times ($P \leq 0.05$; Table 1). The disease intensity on cucumber plants caused by *Alternaria alternata* was least when treated with 1:10 dilution of the extract ($P \leq 0.05$). The 100 times dilution was not significantly effective. The concentrated extract mixture of tender core of banana plant pseudostem and *T. erecta* leaves could significantly reduce the severity of these pathogens on their respective hosts. However, the maximum control was in 1:10 dilution treated plants ($P \leq 0.05$). The extract (1:10


a
b
c
d
Control
Pathogen
E
P + E
P + E (1:10)
P + E (1:100)
P / E (1:1)
P / E (1:10)
P / E (1:100)
E / P (1:1)
E / P (1:10)
E / P (1:100)
0
24
48
72
96
2+ wk
3+ wk
Treatment in hours


a
b
c
d
Enzyme activity (mM min-1 g-1 fresh weight)
Control
Pathogen
E
P + E
P + E (1:10)
P + E (1:100)
P / E (1:1)
P / E (1:10)
P / E (1:100)
E / P (1:1)
E / P (1:10)
E / P (1:100)
Treatment

◀**Fig. 2** Peroxidase specific activity in **a** cucumber plants; **b** spinach plants; **c** tomato plants; and **d** barley plants. *Control* Plants treated with sterilized distilled water (*control*); *pathogen* plants treated with pathogen; *E*: plants treated with extract; $P + E$(1:1): plants treated with premix of pathogen and extract; $P + E$(1:10): plants treated with premix of pathogen and 1:10 dilution of extract; $P + E$(1:100): plants treated with premix of pathogen and 1:100 dilution of extract; *P/E*(1:1): pathogen inoculated 24 h prior to treatment with extract; *P/E*(1:10): pathogen inoculated 24 h prior to treatment with 1:10 dilution of extract; *P/E*(1:100): pathogen inoculated 24 h prior to treatment with 1:100 dilution of extract; *E/P* (1:1): pathogen inoculated after 24 h of treatment with extract; *E/P* (1:10): pathogen inoculated after 24 h of treatment with 1:10 dilution of extract; and *E/P* (1:100): pathogen inoculated after 24 h of treatment with 1:100 dilution of extract (*E*—1:1 mix of aqueous extract of soft inner core of banana pseudostem and leaves of *Tagetes erecta*). No significant change was observed in specific activity in all the treatments

dilution) sprayed prior to their respective pathogen inoculation was most effective in controlling occurrence of the disease.

Significant increase in POX activity in cucumber was observed after 72 h of treatment ($P \leq 0.05$) with undiluted and 1:10 dilution of extract (Fig. 1a). The 100 times diluted extract could not effectively elicit POX activity (Fig. 1a) in cucumber plants. However, the specific activity of the enzyme was unaffected throughout the sampling period (Fig. 2a). As compared to control, acidic POX isoform (Rf = 0.41) was not observed in all the treated samples (Fig. 3a).

In spinach, all the treatments, except the control and pathogen only treated samples, had a significant rise in POX activity ($P \leq 0.05$) after 72–96 h of respective treatment. The undiluted extract could elicit POX activity ($P \leq 0.05$), but the increase was twofold when plants were treated with 1:10 dilution ($P \leq 0.01$). Plants treated with 1:10 dilution of extract after 24 h of pathogen inoculation elicited maximum POX activity (Fig. 1b). Specific activity of POX was slightly higher in plants treated with 1:1 and 1:10 dilution of the extract as compared to control (Fig. 2b). In all the samples, three acidic POX isoforms (Rf 0.02, 0.50 and 0.56) were observed, but when treated with undiluted and 10 times diluted extract, there was an overexpression of POX isoform (Rf 0.50; Fig. 3b).

Tomato plants treated with 1:10 dilution could induce twofold increase in POX activity after 96 h which persisted till 2 weeks of treatment ($P \leq 0.01$; Fig. 1c). 1:100 dilutions were not effective in inducing POX activity. POX isoform (Rf 0.21) was not detected after 96 h of treatment. The enzyme specific activity was almost constant for all the treatments (Fig. 2c). 1:10 dilution treated plants had two new POX isoforms with Rf value 0.35 and 0.38 (Fig. 3c).

Barley plants treated with extracts either simultaneously with pathogen or 24 h post-pathogen inoculation ($P \leq 0.01$) had significant POX activity. The enzyme activity was maximum at 72 h ($P \leq 0.05$; Fig. 1d). Though the specific activity of POX was slightly high in 1:10 and undiluted extract, the increase was not much significant. However, plants treated with 1:100 dilutions had a marginal decrease in enzyme specific activity (Fig. 2d). A new acidic iso-POX (Rf 0.72) was expressed after 72 h of extract application in all dilutions (Fig. 3d).

PPO activity in cucumber significantly increased after 96 h of extract application ($P \leq 0.01$). All the samples had significant ($P \leq 0.05$) increase in PPO activity except in control, pathogen only and 1:100 dilution treated plants (Fig. 4a). There was no visible change in specific activity of PPO in all treatments (Fig. 5a). Six acidic iso-PPOs (Rf 0.28, 0.35, 0.43, 0.44, 0.45 and 0.47) were observed in all the samples. However, 1:10 dilution treated plants had an over expression of one iso-PPO (Rf 0.35) (Fig. 6a).

Significant activity of PPO ($P \leq 0.05$) in all samples of spinach except those treated simultaneously with extract and pathogen was observed after 96 h (Fig. 4b). The specific activity of PPO was almost constant in all treatments (Fig. 5b). The new isoform observed after treatment with undiluted extract was of Rf 0.65. Application of 1:10 diluted extract led to expression of two new isozymes (Rf 0.68 and 0.72; Fig. 6b).

Tomato plants treated with 1:10 dilution of the extract after pathogen inoculation had significantly elicited PPO activity ($P \leq 0.05$) after 96 h of treatment (Fig. 4c). The enzyme did not show any significant change in its specific activity throughout sampling period (Fig. 5c). The zymogram of the gel at 72 h showed overexpression of PPO isozyme (Rf 0.65) in plants treated with the extract (Fig. 6c), whereas plants inoculated with pathogen only had absence of PPO isoforms (Rf 0.72, 0.77).

In barley, a significant ($P \leq 0.05$) increase in the activity of PPO was observed after 72 h of extract application (Fig. 4d). Plants treated with extract 24 h after pathogen inoculation had increased PPO activity. 1:100 times diluted extract did not show any significant change in PPO activity. PPO specific activity was, however, unaffected (Fig. 5d). Plants treated with the extract against pathogen inoculation had expression of an additional iso-PPO (Rf 0.58; Fig. 6d).

Discussion

In the present investigation, an effort was made to understand the efficacy of aqueous extract mix of tender core of banana plant pseudostem and *T. erecta* leaves as a biocontrol agent and its impact on physiology of host defense. The study is important since any alteration in activity of POX and PPO, the vital enzymes of host defense physiology, would affect the interaction between the host and

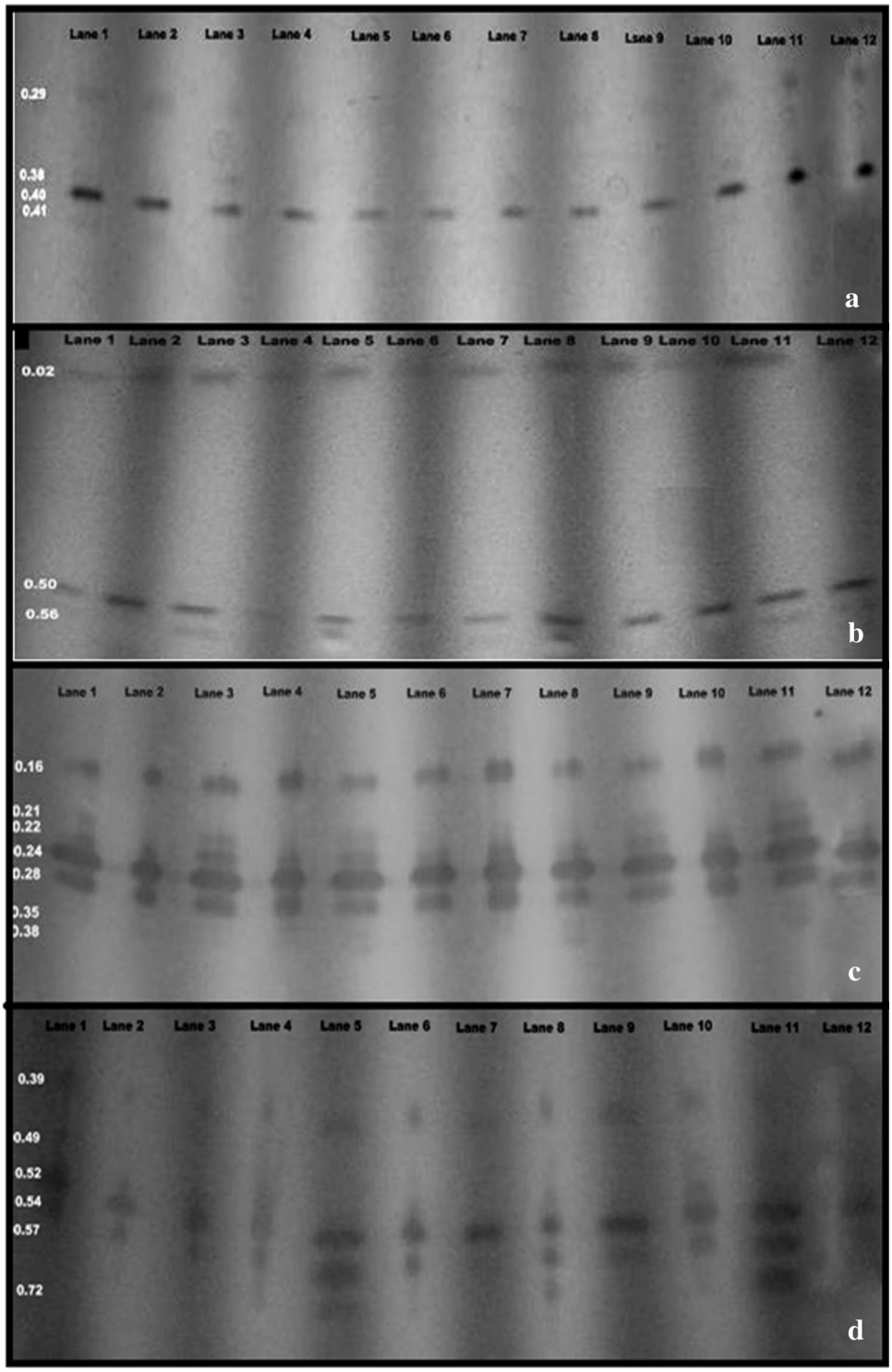


Fig. 3 Isozymes of peroxidase in **a** cucumber plants after 72 h of extract application; **b** spinach plants after 72 h of extract application; **c** tomato plants after 96 h of extract application; and **d** barley plants after 72 h of extract application. *Lane 1* plants treated with sterilized distilled water (control). *Lane 2* plants treated with pathogen. *Lane 3* plants treated with extract. *Lane 4* plants treated with premix of pathogen and extract. *Lane 5* plants treated with premix of pathogen and 1:10 dilution of extract. *Lane 6* plants treated with premix of pathogen and 1:100 dilution of extract. *Lane 7* pathogen inoculated 24 h prior to treatment with extract. *Lane 8* pathogen inoculated 24 h prior to treatment with 1:10 dilution of extract. *Lane 9* pathogen inoculated 24 h prior to treatment with 1:100 dilution of extract. *Lane 10* pathogen inoculated after 24 h of treatment with extract. *Lane 11* pathogen inoculated after 24 h of treatment with 1:10 dilution of extract. *Lane 12* pathogen inoculated after 24 h of treatment with 1:100 dilution of extract

the pathogen which can have a tremendous impact on spread and control of potent pathogens on crop plants.

Several studies have reported the role of plant extracts in possessing antibacterial and antifungal properties. Wang et al. [29] demonstrated the effective role of neem seed kernel extracts against fungal pathogens in plum and Yali fruits. Mbega et al. [30] observed that extracts from *Aloe vera, Betula pendula, Coffea arabica, Ocimum basilicum* and *Salvia officinalis* could completely inhibit *Xanthomonas perforans* in both in vitro and *in planta* assays in tomato. *Clerodendrum japonicum* and *Catharantus roseus* leaf extract could induce SAR through salicylic acid phenylpropanoid pathway in tomato cultivars infected by CMV virus [31]. The extracts of *Ocimum basilicum*,

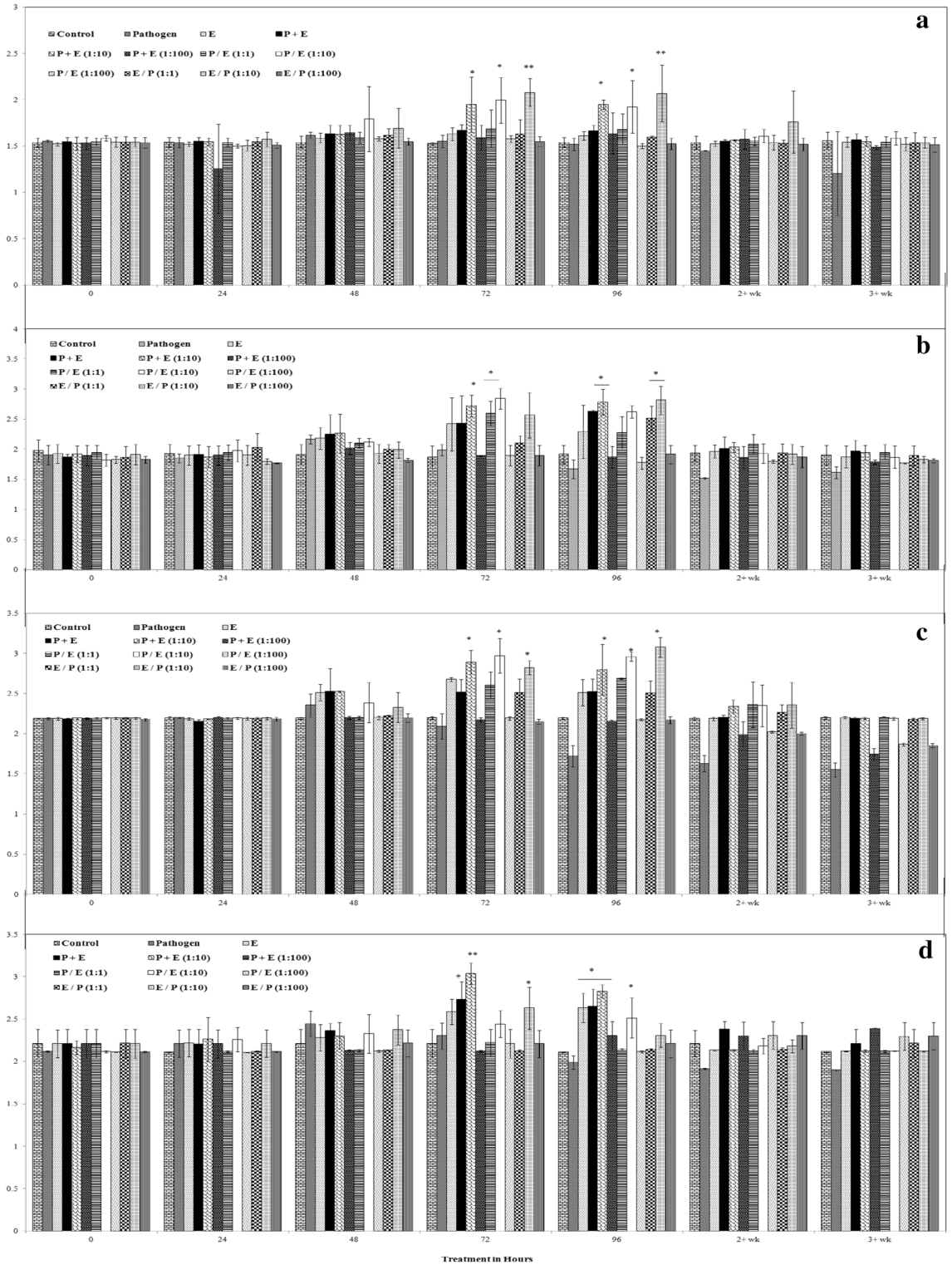


Fig. 4 Polyphenol oxidase activity in **a** cucumber plants; **b** spinach plants; **c** tomato plants; and **d** barley plants. ▦: Plants treated with sterilized distilled water (control); ▩: plants treated with pathogen; ⁘: plants treated with extract; ■: plants treated with premix of pathogen and extract; ▫: plants treated with premix of pathogen and 1:10 dilution of extract; ▪: plants treated with premix of pathogen and 1:100 dilution of extract; ▬: pathogen inoculated 24 h prior to treatment with extract; □: pathogen inoculated 24 h prior to treatment with 1:10 dilution of extract; ⁘: pathogen inoculated 24 h prior to treatment with 1:100 dilution of extract; ▚: pathogen inoculated after 24 h of treatment with extract; ▤: pathogen inoculated after 24 h of treatment with 1:10 dilution of extract; and ▥: pathogen inoculated after 24 h of treatment with 1:100 dilution of extract. $*p \leq 0.05$; $**p \leq 0.01$. $^{\Psi}$*Vertical bars* represent standard error

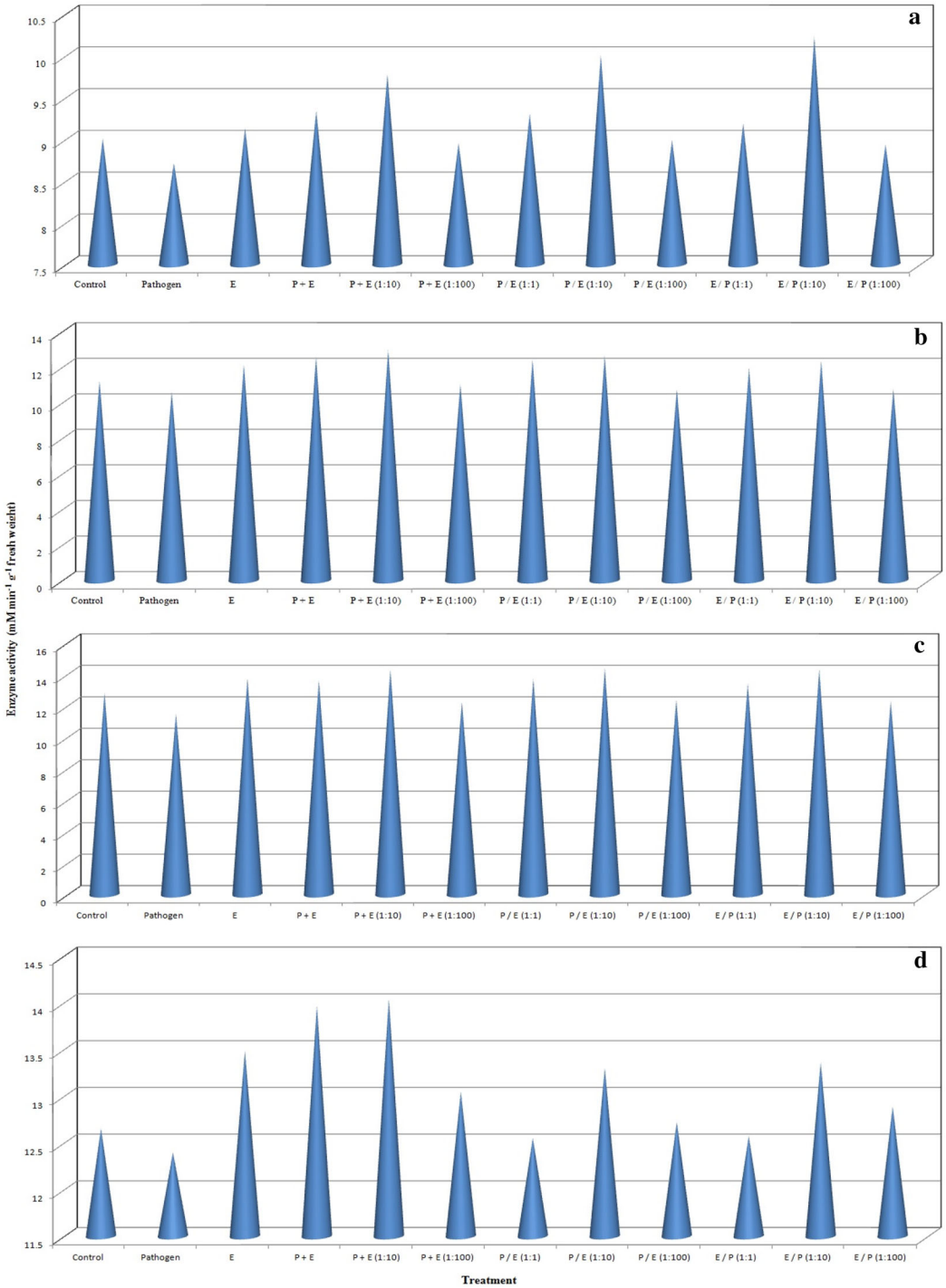


Fig. 5 Polyphenol specific activity in **a** cucumber plants; **b** spinach plants; **c** tomato plants; and **d** barley plants. *Control* plants treated with sterilized distilled water (control); *Pathogen* plants treated with pathogen; **e** plants treated with extract; $P + E(1{:}1)$: Plants treated with premix of pathogen and extract; $P + E(1{:}10)$: plants treated with premix of pathogen and 1:10 dilution of extract; $P + E(1{:}100)$: plants treated with premix of pathogen and 1:100 dilution of extract; *P/E*(1:1): pathogen inoculated 24 h prior to treatment with extract; *P/E*(1:10): pathogen inoculated 24 h prior to treatment with 1:10 dilution of extract; *P/E*(1:100): pathogen inoculated 24 h prior to treatment with 1:100 dilution of extract; *E/P* (1:1): pathogen inoculated after 24 h of treatment with extract; *E/P* (1:10): pathogen inoculated after 24 h of treatment with 1:10 dilution of extract; and *E/P* (1:100): pathogen inoculated after 24 h of treatment with 1:100 dilution of extract. (*E*—1:1 mix of aqueous extract of soft inner core of banana pseudostem and leaves of *Tagetes erecta*). No significant change was observed in specific activity in all the treatments

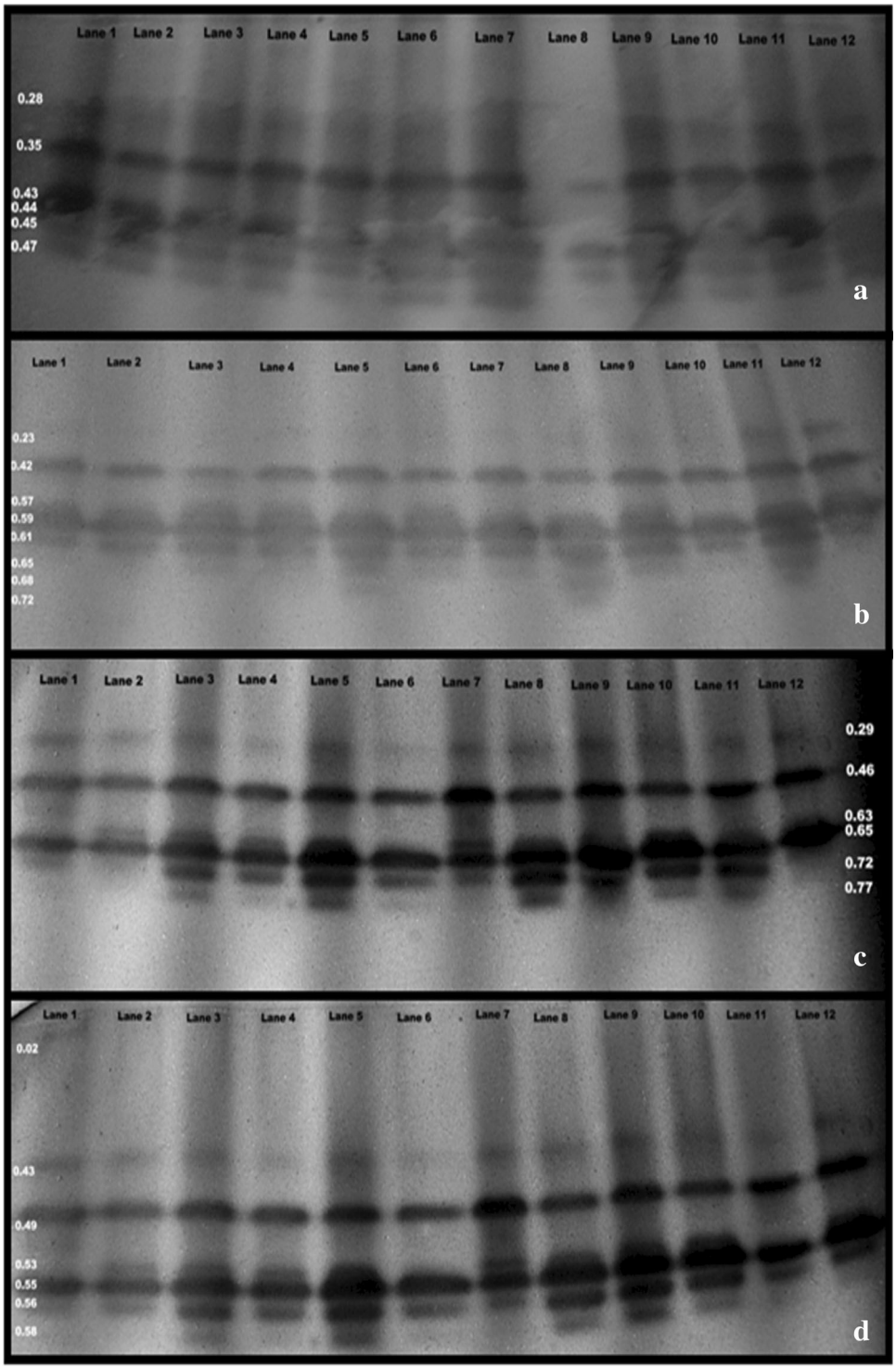


Fig. 6 Isozymes of polyphenol oxidase in **a** cucumber plants after 96 h of extract application; **b** spinach plants after 96 h of extract application; **c** tomato plants after 72 h of extract application; **d** barley plants after 72 h of extract application. *Lane 1* plants treated with sterilized distilled water (control). *Lane 2* plants treated with pathogen. *Lane 3* plants treated with extract. *Lane 4* plants treated with premix of pathogen and extract. *Lane 5* plants treated with premix of pathogen and 1:10 dilution of extract. *Lane 6* plants treated with premix of pathogen and 1:100 dilution of extract. *Lane 7* pathogen inoculated 24 h prior to treatment with extract. *Lane 8* pathogen inoculated 24 h prior to treatment with 1:10 dilution of extract. *Lane 9* pathogen inoculated 24 h prior to treatment with 1:100 dilution of extract. *Lane 10* pathogen inoculated after 24 h of treatment with extract. *Lane 11* pathogen inoculated after 24 h of treatment with 1:10 dilution of extract. *Lane 12* pathogen inoculated after 24 h of treatment with 1:100 dilution of extract

Azadirachta indica, *Eucalyptus chamadulonsis, Datura stramonium*, *Nerium oleander* and *Allium sativum* could significantly reduce disease severity in tomato plants infected with *A. solani* (32). In the present study, mix of aqueous extracts from banana pseudostem and marigold leaves was used for testing their efficacy in reducing disease severity in four plant-pathogen systems. Similarly, several studies have been reported on the role of these plant extracts in possessing antibacterial and antifungal properties. Meenashree et al. [33] found that ethanolic, acetone and petroleum ether extracts of fresh banana leaf had antimicrobial properties against pathogenic fungi *Aspergillus terreus* and *Penicillium solitum*. Mordi et al. [34] isolated lectin from banana fruit and found it to possess anti

HIV-1 activity. Water extract of banana core has been reported to control pathogens in a broad range of plants. Jahan et al. [35] and Jain et al. [36] found the extract of banana (*Musa sapientum*) very effective against several Gram-positive and Gram-negative bacteria. No study has been reported using the tender core of banana stem.

Marigold (*Tagetes* spp.) secretes toxic metabolites like α-terthienyl, polyacetylenes and polythienyls, substances well known for their insecticidal and nematicidal properties [37, 38]. Debprasad et al. [39] and Hussain et al. [40] reported that *T. erecta* contains compounds like dodecanoic acid, myristic acid, palmitic acid, stearic acid, myristoleic acid and tricosane which show nematicidal activity against *M. incognita*. Our studies demonstrated the inhibitory properties of marigold leaf extracts against pathogenic bacteria and fungi. Seven coumarins and other compounds isolated from *Tagetes* spp. were assayed against pathogenic bacteria and fungi [41]. Total extract and fractions obtained with different solvents from leaves of *Tagetes minuta* showed several degrees of antimicrobial activity against Gram-positive and Gram-negative microorganisms [42]. Dasgupta et al. [43] reported the antibacterial effect of *Tagetes erecta* leaf extract at against both Gram-positive and Gram-negative bacterial clinical pathogen isolates.

The banana-marigold crude extract mix could significantly induce POX and PPO activities. The aqueous extract of tender core of banana pseudostem and *T. erecta* leaves significantly influenced the expression and activity of POX and PPO enzymes and can play a major role in controlling plant diseases. This is evidenced from the observation that the activity enhancement of POX and PPO occurs within 72–96 h of treatment. The disease intensity and disease severity are also significantly reduced when plants are treated with the crude extract.

In the current study, the experiments were conducted under glasshouse conditions. The aqueous extracts of banana pseudostem and *Tagetes* leaves could thus be formulated into an environmentally friendly biocide agent against a wide range of plant pathogens. The study can be extended for field trials on a range of crops and pathogens including banana plantations.

Acknowledgements The authors express their sincere thanks to Amity Institute of Biotechnology, Amity University Uttar Pradesh, NOIDA, India, for providing the necessary infrastructural facilities and DST–INSPIRE, Government of India, for providing Senior Research Fellowship to the first author.

References

1. Walters, D. (2011). *Plant defense: warding off attack by pathogens, herbivores and parasitic plants*. New York: Wiley.
2. Aman, M., & Rai, V. R. (2015). Antifungal activity of fungicides and plant extracts against yellow sigatoka disease causing *Mycosphaerella musicola*. *Current Research in Environmental and Applied Mycology, 5,* 277–284.
3. Gupta, S., & Dikshit, A. K. (2010). Biopesticides: An eco-friendly approach for pest control. *J biopesticides, 3,* 186–188.
4. Kumar, K. S., Bhowmik, D., Duraivel, S., & Umadevi, M. (2012). Traditional and medicinal uses of banana. *Journal of Pharmacognosy and Phytochemistry, 1*(3), 51–63.
5. Mokbel, M. S., & Hashinagam, F. (2005). Antibacterial and antioxidant activities of banana (*Musa*, AAA cv. Cavendish) fruits peel. *American Journal of Biochemistry and Biotechnology, 1*(3), 125–131.
6. Fagbemi, J. F., Ugoji, E., Adenipekun, T., & Adelowotan, O. (2009). Evaluation of the antimicrobial properties of unripe banana (*Musa sapientum* L.), lemon grass (*Cymbopogon citratus* S.) and turmeric (*Curcuma longa* L.) on pathogens. *African Journal of Biotechnology, 8*(7), 1176–1182.
7. Ehiowemwenguan, G., Emoghene, A. O., & Inetianbor, J. E. (2014). Antibacterial and Phytochemical analysis of banana fruit peel. *IOSR Journal of Pharmacy, 4*(8), 18–25.
8. Bakker, J., Gommers, F. J., Nieuwenhuis, I., & Wynberg, H. (1979). Photoactivation of the nematicidal compound alpha–terthienyl from roots of marigolds (*Tagetes* species). A possible singlet oxygen role. *Journal of Biological Chemistry, 254*(6), 1841–1844.
9. Hooks, C. R., Wang, K. H., Ploeg, A., & McSorley, R. (2010). Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant–parasitic nematodes. *Applied Soil Ecology, 46*(3), 307–320.
10. Maradufu, A., Lubega, R., & Dom, F. (1978). Isolation of (5E)-Ocimerone, a mosquito larvicide from *Tagetes minuta*. *Lloydia (Cincinnati), 41,* 181–183.
11. Weaver, D. K., Wells, D., Dunkel, F. V., Bertsch, W., Sing, S. E., & Sriharan, S. (1994). Insecticidal activity of floral, foliar, and root extracts of *Tagetes minuta* (Asterales: Asteraceae) against adult Mexican bean weevils (Coleoptera: Bruchidae). *Journal of Economic Entomology, 87*(6), 1718–1725.
12. Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S., & Dhakshnamoorthy, G. (2006). Cold aqueous extracts of African marigold, *Tagetes erecta* for control tomato root knot nematode, Meloidogyne incognita. *Crop Protection, 25*(11), 1210–1213.
13. Şesan, T. E., Enache, E., Iacomi, B. M., Oprea, M., Oancea, F., & Iacomi, C. (2015). Antifungal activity of some plant extracts against Botrytis cinerea Pers. in the blackcurrant crop (Ribes nigrum L.). *Acta Scientiarum Polonorum Hortorum Cultus, 14,* 29–43.
14. Karuppiah, P., & Mustaffa, M. (2013). Antibacterial and antioxidant activities of *Musa* sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. *Asian Pacific Journal of Tropical Biomedicine, 3*(9), 737–742.
15. Naikwade, P. V., Gaurav, S., Sharayu, D., & Kailas, J. (2014). Evaluation of antibacterial properties of Musa paradisiaca L. Leaves. In *Proceeding of the national conference on conservation of natural resources & biodiversity for sustainable development.*
16. Singh, G., Singh, O. P., De Lampasona, M. P., & Catalan, C. A. (2003). Studies on essential oils. Part 35: chemical and biocidal investigations on *Tagetes erecta* leaf volatile oil. *Flavour and Fragrance Journal, 18*(1), 62–65.
17. Dharmagadda, V. S. S., Tandonb, M., & Vasudevan, P. (2005). Biocidal activity of the essential oils of *Lantana camara*, *Ocimum sanctum* and *Tagetes patula*. *Journal of Scientific and Industrial Research, 64*(1), 53–56.
18. Politi, F. A., Queiroz-Fernandes, G. M., Rodrigues, E. R., Freitas, J. A., & Pietro, R. C. (2016). Antifungal, antiradical and cytotoxic activities of extractives obtained from Tagetes patula L. (Asteraceae), a potential acaricide plant species. *Microbial Pathogenesis, 95,* 15–20.

19. Sangeetha, M., Rajendran, S., Sathiyabama, J., & Prabhakar, P. (2012). Eco friendly extract of Banana peel as corrosion inhibitor for carbon steel in sea water. *Journal of Natural Product and Plant Resources, 2*(5), 601–610.
20. Haq, R. A., Hussain, M., Cheema, Z. A., Mushtaq, M. N., & Farooq, M. (2010). Mulberry leaf water extract inhibits bermudagrass and promotes wheat growth. *Weed Biology and Management, 10*(4), 234–240.
21. Yunis, H., Bashan, Y., Okon, Y., & Henis, Y. (1980). Two sources of resistance to bacterial speck of tomato caused by *Pseudomonas* tomato. *Plant Disease, 64,* 851–852.
22. Arabi, M. I. E., & Jawhar, M. (2010). Interrelationship between incidence and severity of leaf stripe on barley. *Journal of Plant Pathology, 92,* 503–505.
23. Mitra, J., Bhuvaneshwari, V., & Paul, P. K. (2013). Broad spectrum management of plant diseases by phylloplane microfungal metabolites. *Archives of Phytopathology and Plant Protection, 46*(16), 1993–2001.
24. Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analytical Biochemistry, 53,* 304–308.
25. de Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., Abreu, C. E. B. D., & Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany, 56*(1), 87–94.
26. Rao, K. V., Suprasanna, P., & Reddy, G. M. (1989). Studies on enzyme and isozyme patterns in embryogenic glume calli of maize. *Indian National Science Academy, 4,* 277–280.
27. Bogdanović, J., Dučić, T. M., Milosavić, N. B., Vujčić, Z., Šijačić, M., Isajev, V., et al. (2005). Antioxidant enzymes in the needles of different omorika lines. *Archives of Biological Science, 57,* 277–282.
28. Anand, T., Raguchander, T., Karthikeyan, G., Prakasam, V., & Samiyappan, R. (2007). Chemically and biologically mediated systemic resistance in cucumber (*Cucumis sativus* L.) against *Pseudoperonospora cubensis* and *Erysiphe cichoracearum*. *Phytopathologia Mediterranea, 46*(3), 259–271.
29. Wang, J., Li, J., Cao, J., & Jiang, W. (2010). Antifungal activities of neem (*Azadirachta indica*) seed kernel extracts on postharvest diseases in fruits. *African Journal of Microbiology Research, 4*(11), 1100–1104.
30. Mbega, E. R., Mortensen, C. N., Mabagala, R. B., & Wulff, E. G. (2012). The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. *Journal of General Plant Pathology, 78*(4), 277–286.
31. Rahmawati, S. L., Esyanti, R. R., & Gunaeni, N. (2014). The role of leaf extracts as plant-activator to enhance salicylic acid production on tomato plant (*Lycopersicon esculentum* Mill.) Infected by CMV (Cucumber Mosaic Virus). International Journal of Chemical, Environmental and Biological Sciences, *2*. www.isaet.org.
32. Nashwa, S. M., & Abo-Elyousr, K. A. (2012). Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science, 48*(2), 74–79.
33. Meenashree, B., Vasanthi, V. J., & Mary, R. N. I. (2014). Evaluation of total phenolic content and antimicrobial activities exhibited by the leaf extracts of *Musa acuminata* (banana). *International Journal of Current Microbiology and Applied Sciences, 3*(5), 136–141.
34. Mordi, R. C., Fadiaro, A. E., Owoeye, T. F., Olanrewaju, I. O., Uzoamaka, G. C., & Olorunshola, S. J. (2016). Identification by GC-MS of the components of oils of banana peels extract, phytochemical and antimicrobial analyses. *Research Journal of Phytochemistry*. doi:10.3923/rjphyto.2016.
35. Jahan, M., Warsi, M. K., & Khatoon, F. (2010). Concentration influence on antimicrobial activity of banana blossom extract-incorporated chitosan-polyethylene glycol (CS-PEG) blended film. *Journal of Chemical and Pharmaceutical Research, 2*(5), 373–378.
36. Jain, P., Bhuiyan, M. H., Hossain, K. R., & Bachar, S. C. (2011). Antibacterial and antioxidant activities of local seeded banana fruits. *African Journal of Pharmacy and Pharmacology, 5*(11), 1398–1403.
37. D'Addabbo, T., Laquale, S., Lovelli, S., Candido, V., & Avato, P. (2014). Biocide plants as a sustainable tool for the control of pests and pathogens in vegetable cropping systems. *Italian Journal of Agronomy, 9*(4), 137–145.
38. Tripathi, P., Shukla, A. K., Ganesan, S., Vadivel, K., & Jayaraman, J. (2015). Potential use of essential oils, plant fats and plant extracts as botanical fungicides (p. 19). Sustainable Crop Disease Management using Natural Products, CAB International.
39. Debprasad, R., Prasad, D., Singh, R. P., & Ray, D. (2000). Chemical examination and antinemic activity of marigold (*Tagetes erecta* L) flower. *Annals of Plant Protection Sciences, 8*(2), 212–217.
40. Hussain, M. A., Mukhtar, T., & Kayani, M. Z. (2011). Efficacy evaluation of *Azadirachta indica, Calotropis procera, Datura stramonium and Tagetes erecta* against root-knot nematodes *Meloidogyne incognita*. *Pakistan Journal of Botany, 43*(1), 197–204.
41. Céspedes, C. L., Avila, J. G., Martínez, A., Serrato, B., Calderón-Mugica, J. C., & Salgado-Garciglia, R. (2006). Antifungal and antibacterial activities of Mexican tarragon (*Tagetes lucida*). *Journal of Agriculture and Food Chemistry, 54*(10), 3521–3527.
42. Tereschuk, M. L., Riera, M. V., Castro, G. R., & Abdala, L. R. (1997). Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. *Journal of Ethnopharmacology, 56*(3), 227–232.
43. Dasgupta, N., Ranjan, S., Saha, P., Jain, R., Malhotra, S., & Arabi Mohamed Saleh, M. A. (2012). Antibacterial activity of leaf extract of Mexican marigold (*Tagetes erecta*) against different gram positive and gram negative bacterial strains. *Journal of Pharmacy Research, 5*(8), 4201–4203.