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Original Research Article

Assessment of genotoxic effects of pesticide and vermicompost treated soil with *Allium cepa* test

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ABSTRACT

Soil forms a huge reservoir of nutrients that sustains life on earth. Anthropogenic and natural impacts have led to degradation of land which declines the overall quality of soil, water or vegetation. The present study involves comparison of genotoxicity of soil procured from two different agricultural sites, pesticide treated soil (PTS) and vermicompost treated soil (VTS). The soil was physico-chemically characterized and showed significant differences in terms of cytotoxicity (root length; mitotic index) and genotoxicity (chromosomal aberrations) in *Allium cepa* test. The mitotic index of the control after 24 and 48 h was found to be 26.1 ± 1.6 and 26.1 ± 1.3 respectively. Mitotic index was reduced to 10.3 ± 0.9 and 9.7 ± 0.6 in 100% PTS and 24.4 ± 1.7 and 25.4 ± 0.8 in 100% VTS after 24 and 48 h of exposure, respectively. Clastogenic aberrations were found to be highest (54.5%) in 100% PTS which was significantly different from VTS extract. The PTS extracts incurred significantly more cytotoxic and genotoxic effects on *A. cepa* in comparison to VTS. The result indicates that addition of vermicompost in agriculture field acts as soil ameliorator and plays an important role in promotion of cell division and proliferation, hence good for the plant health and crop productivity.

1. Introduction

The escalation of industrial as well as agricultural enactment substantially the use of pesticides has made our natural resources absolutely sunken and has severely affected the biodiversity and ultimately soil health. Concern has been raised over the long term sustainability and environmental consequences due to intensification of agro-ecosystem. In Punjab, the intensive rice-wheat systems have started to decipher the signs of serious decline associated with loss of soil quality and increased plant health problems [1]. Pesticides enter the soil by direct supplementation (agricultural practices) or by indirect methods (discharge from production sites, accidental spillage, leakage from pesticide dump sites, surface run off, etc.). Pesticides are known to affect the behavior of soil enzymatic system that plays an important role in soil-plant biological activity [2]. The residues of pesticides prevalent in environmental matrices are of larger concern [3]. Fertilizer and pesticide usage tend to elevate the level of nutrients in soil and simultaneously increasing toxins in water and soil. Excessive use of pesticides and fertilizers also pollute the surrounding environment in addition to deteriorating the physical and chemical properties of soil [4]. The usage of such pesticides and fertilizers often leads to a reduction in total microbial activity, porosity, particle and bulk density of soil [4] and excessive leaching of nutrients resulting in salinity induced plant stress [5,6].

Vermicompost on the other hand is basically a complex mixture of fecal matter of earthworm's humified organic matter and microorganisms. Supplementation of vermicompost in soil or plant growth media leads to increased germination, elevates growth, flowering, and accelerates the production of fruit. Vermicompost helps in maintaining the soil structure healthy by altering the physico-chemical properties of soil [7]. Vermicompost plays an important role in increasing the size, biodiversity and activity of microbes in the soil [4]. It is also a great source of nutrients and

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organic matter and retains it for a longer period of time without any harmful effect on the environment [8]. It contains high level of plant growth hormones and soil enzymes [9]. Usage of vermicompost may lead to fine tuning of input nutrients and plant needs and thus has maximized yields.

Allium cepa is used as a test material to determine genotoxic effects of different chemicals. Environmental agencies like the United Nations Environmental Program, World Health Organization, International Program on Plant Bioassay and US Environmental Protection Agency have advocated and validated the use of plants as test organisms [10]. *Allium* genus, especially the use of *A. cepa* for bio-monitoring of genotoxicity is considered to be very efficient [11]. This assay has been utilized to detect genotoxicity of various products like pressmud, waste water sludge, distillery sludge, etc. The present paper has embarked on two major aspects: (i) the effect of pesticides on the genotoxicity of *A. cepa* and (ii) the effect of vermicompost on *A. cepa.* This study also focuses on the dynamics of the pesticides present in the agricultural farmlands that are deteriorating the crop quality. Altogether, there is a dire need of a healthy ecosystem that provides with fertile soil, clean water, food and other natural resources [9]. Thus, the evaluation of agricultural soils for detection of their potency to risk biodiversity and ecological systems is obligatory. In this approach the effect of the bioavailable fractions of pollutants/chemicals present in the complex soil matrix was taken into account.

2. Materials and methods

2.1. Site for collection of samples

The pesticide treated soil (PTS) samples were collected from various sites of agriculture field in district Phagwara, Punjab, India (31°13′4″ N latitude and 75°46′9″ E longitude) under maize cultivation where herbicide Atrataf 50 WP (atrazine) @ 2.47×10^{-4} kg m^{-2} was sprayed. The vermicompost treated soil (VTS) samples were collected from organic farmland where pulses (mung bean) were grown and vermicompost was applied @ 0.123 kg m^{-2}. Soil samples were collected from 4 to 5 sites of each agricultural field from the depth of 0.15–0.2 m and pooled together to make a single sample. Samples were brought to laboratory and dried at room temperature for 72 h. It was then finally ground to fine powder, sieved and saved for further investigation.

2.2. Physico-chemical analysis

Both the soil samples were collected and analyzed for physico-chemical parameters like pH, electrical conductivity (EC), total dissolved solids (TDS), total available phosphorus (TAP), total potassium (TK), total organic carbon (TOC), total organic matter (TOM), total sodium (TNa), total calcium (TCa), total lithium (TLi). The texture of the soil samples was determined using a hydrometer. The initial reading was taken at 4 min and 48 s and final after 5 h. The clay, silt and sand content were calculated from this. pH, EC and TDS were calculated over digital meter (Eutech Instruments, PCSTestr 35 series). For this, 5 g of soil (air-dried) was suspended in 50 mL of distilled water (1:10 w/v) in a flask. The contents were kept on an orbital shaker for 30 min and pH, EC and TDS were determined from the supernatant. The air dried samples were digested to analyze TAP, TK, TNa, TCa and TLi. 0.5 g of soil sample was taken and digested with di-acidic mixture ($HClO_4$/HNO_3 in a 4:1 ratio). TAP was then calculated by the method described by John [12] using Systronics double beam spectrophotometer 2202. Absorbance was recorded at 882 nm. The other elements TK, TNa, TCa and TLi were measured by Systronics flame photometer-128. Standard solutions were prepared by using respective salts of the estimated elements. The TOC and TOM were calculated by Walkley Black method [13]. Barium diphenylalanine sulphonate was used as an indicator and $FeSO_4$ as the titrant.

2.3. Allium for genotoxicity tests

Equal sized bulbs of *A. cepa* were purchased from the local market. Dried and mold attack onion bulbs should be discarded and onions must not have started shooting green leaves. The onions were submerged in water for 10–12 h so as to soften their scales. The outer scales were then removed carefully without damaging the root primordials. After the removal of outer scales, the bulbs were kept into fresh water to prevent the root primordial from drying. Ten onion bulbs were set up for each concentration. The experiment was performed at about 20 ± 2 °C and was protected against direct sun light. The bulbs were placed in distilled water and kept undisturbed for 3 d till roots of 1–2 cm length were obtained. The bulbs were then treated with various concentrations of PTS and VTS for 24 and 48 h. After 24 and 48 h, the root length was measured. The treatment solution was changed after every 24 h [14].

2.4. Preparation of extract

The extracts of VTS and PTS were prepared according to the French Standardized Method [15]. In brief, 1 L of water was added to 100 g of soil sample which was then subjected to continuous shaking for 24 h. After that the suspension was filtered using a whatman Filter Paper 42 (pore size 2.5 μm). The filtrate was evaluated further for genotoxic effects immediately. Various concentrations (10, 20, 40, 60, 80 and 100%) of extracts along with negative control (distilled water) were used for treatment.

2.5. Fixation, slide preparation and scoring

After treatment, the root tips were excised, washed and then fixed in Farmer's fluid (glacial acetic acid and ethanol in 1:3 ratio). After 24 h of fixation, the root tips were hydrolyzed in 1 N HCl for 2–3 min and then squashed in aceto-carmine and 1 N HCl (9:1) in water bath (60 °C) for 12–15 min. The meristematic zone was removed and immersed in 45% glacial acetic acid for 1 min and then transferred to a clean slide, squashed under a cover slip and sealed with mountant DPX (a mixture of distyrene, a plasticizer (tricresyl phosphate) and xylene). All slides were coded and examined under a light microscope.

2.6. Toxicity studies

Root length is estimated in whole root bundles [14]. The mean value is calculated from ten measurements and relative growth value is expressed as percent of the control value. Other signs of toxicity like change in color of roots, consistency of roots, presence of root hooks, twists, or crochets were also examined. The comparison of toxic effects was analyzed by calculating the mitotic index (MI) and chromosomal aberrations (CA). The MI was determined by the examination of number of dividing cells for each concentration along with the total number of diving cells. The MI is a measure of the proliferation status of a cell population. It is defined as the ratio between the number of cells undergoing mitosis and the total number of cells. CA, on the other hand is any irregularity or abnormality in the number and structure of chromosomes. The CA was also observed and classified as physiological aberrations (PA) and clastogenic aberrations (CGA). The PA includes c-mitosis, stickiness, vagrant chromosomes, laggard chromosomes and CGA includes chromatin bridges and chromosomal breaks. The

CA was calculated out of 1000 cells for every percent concentration of each treatment.

2.7. Statistical analysis

The differences between the physico-chemical parameters for both the soils were evaluated by Student's unpaired *t*-test. The level of significance for the MI and the root length were also evaluated by Student's *t*-test. The linear relationship between CA and various concentrations was obtained by correlation and regression analysis. Statistical analysis was done with the help of Minitab version 14.0 (Pennsylvania, USA) and IBM SPSS version 16.0 (Chicago, USA) computer software programs. The experimental data are presented as mean ± SD of replicate experiment.

3. Results and discussion

Physico-chemical analysis of both the soil types was performed. Toxic effects of PTS and VTS were evaluated in terms of macroscopic and microscopic parameters.

3.1. Physico-chemical analysis of soil

Significant difference was observed between the two soil types with respect to physico-chemical parameters (Table S1 in Supplemental Material). In VTS the pH (7.63) was recorded to be significantly higher ($p < 0.05$) than the PTS (7.32) although both were alkaline in nature. The increase in pH may be the result of the decomposition of nitrogenous substances during vermicomposting [16]. Earthworms also support the population build up of catabolically active microbes [17] which results into degradation of short chain fatty acids and precipitation of calcium carbonate which may have lead to increase in pH of VTS. However it has also been reported that pH is dynamic and substrate dependent [18]. Humus is also reported to bind free cations and raise pH of the soil [19] which may be another reason for a higher pH of the products of vermicomposting. The EC reflects the salinity of a material and is a good indicator of applicability and utility of compost or vermicompost in agricultural purposes. The EC decreased significantly ($p < 0.001$) from PTS to VTS. This decrease can be attributed to the stabilization of the mixtures and reduction of ions. This was corroborated with the findings while studying the vermicomposting of pressmud [17]. TDS also showed similar trend as EC ($p < 0.001$). The TOM was also significantly different ($p < 0.001$). TOM in case of VTS was 1.5 folds higher than PTS. TOC was found to be significantly higher ($p < 0.001$) in VTS in comparison to PTS. TOC in case of VTS was 4.9 while in that of PTS was 3.3. Several studies support these results explaining about carbon loss from substrates in the form of CO_2 brought out by the combined action of earthworms and microbes [20]. Reduction of TOC during vermicomposting in sugar industry waste; municipal waste; sugar mill sludge was also reported [10,21]. TAP showed no significant difference with respect to the samples VTS and PTS. The gut enzymes of earthworms stimulate the phosphate solubilizing microbes thereby promoting the release of phosphorus in vermicast [21]. An increase in phosphate content of vermicompost, which was due to presence of acid and alkaline phosphatases in the worm gut was also observed [22]. However the level of phosphorus in PTS can be attributed to the use of phosphate solubilizing fertilizers and organophosphorus pesticides. TK significantly ($p < 0.001$) declined from PTS to VTS. In VTS, TK was decreased by 53%. Similar decline in level of potassium during the vermicomposting of textile mill sludge and solid waste from leather industry was also noted. This decline was attributed to the use of potassium by earthworms during metabolic activity [17]. Higher pH is also known to make potassium ions more susceptible to fixation by colloids and thus a rise in pH is responsible for decline in level of potassium [19]. TNa decreased significantly ($p < 0.001$) from PTS to VTS. The percent decrease was (49%). This was supported by Bhat et al. [17] but Subramanian et al. [23] observed that sodium content remained unchanged during the vermicomposting of sago industry solid wastes. TCa level was found to be significantly ($p < 0.001$) higher in VTS than PTS. The TCa level in VTS was 95% higher than PTS. Many other studies supported these findings [24] but low content of Ca in cast compared to surrounding soil was also reported [25]. Earthworms promote the process of mineralization converting a proportion of bound form of calcium to free forms, resulting in its enrichment [24]. There was no significant difference in the level of TLi with respect to VTS and PTS.

3.2. Macroscopic parameters

A. cepa test is a standard test for rapid and sensitive screening of chemicals and pollutants that represent environmental hazards. Root tip is often the first and foremost part of a plant that comes into contact with chemicals/pollutants found in water or soil. Root tip system of *A. cepa* has particularly shown sensitivity to harmful effects of environmental hazards [17]. The effects were observed after 24 and 48 h. However there are several studies that indicate toxic effects of various chemicals with *A. cepa* test in less than 24 h even in just 3 h [10]. The effects may be observed by analyzing macroscopic parameters like root growth and root shape or by microscopic parameters. The root lengths of *A. cepa* after exposure to various concentrations of VTS and PTS were significantly different after 24 and 48 h (Table 1). The maximum root length was observed in 10% PTS after 24 h and then in 10% VTS concentration after 48 h. The root length and concentration were negatively correlated in both cases of PTS after exposure of 24 h ($r = -0.74$, $n = 6$, $P < 0.05$) and 48 h ($r = -0.99$, $n = 6$, $P < 0.05$) and in case of VTS after 24 h ($r = -0.96$, $n = 6$, $P < 0.05$) and 48 h ($r = -0.90$, $n = 6$, $P < 0.05$). The average decrease in root length was far more prominent in PTS extracts concentrations than in VTS and significant difference in root lengths were found in 20, 40 and 80% concentrations of PTS and VTS. It was observed that PTS extracts suppressed root growth when compared with VTS extracts. Thus, VTS shows ameliorated effects than PTS. The presence of twists (crochet, hooks) in root was noticed in higher concentration of PTS after 48 h whereas VTS showed normal growth of roots in all concentrations. The regulation of root growth is brought together by independent events that lead to the process of cell division in the mitotically active meristematic zone and cell elongation in proximal region of the root tip. Inhibition of root development and the appearance of stunted roots are indicators of growth retardation and cytotoxicity [26].

3.3. Microscopic parameters

Growth retardation in *A. cepa* is explained by cytotoxicity and chromosomal anomalies as genotoxicity. Toxicity is not always related to genotoxicity. The parameter of cytotoxicity is reliable, quick and sensitive enough for monitoring even slightly polluted surface waters. This study evaluated the potential application of plant genotoxicity tests for scanning mutagens in agricultural soils. Several other studies have been carried out using this bioassay to evaluate the genotoxicity of pesticides like monocrotophos and chlorpyriphos [27], malathion [28]; industrial waste [29] and agricultural residue [30]. The data of mutagenicity assays and plant genotoxicity are considered to be of limited value by regulatory agencies when extrapolated to humans; although they do accept data of non-mammalian systems like bacteria, yeast, and *Drosophila* species. But it has been found that certain chemicals give

Table 1
Comparative root length (mean ± standard deviation) of *A. cepa* exposed to various concentrations of VTS and PTS extracts after 24 and 48 h.

Concentrations	Exposure time (h)	VTS		PTS	
		Root length (cm)	% Root length	Root length (cm)	% Root length
Control	24	3.51 ± 0.34	100.0	3.51 ± 0.34	100.0
	48	4.22 ± 0.40	100.0	4.22 ± 0.40	100.0
10%	24	3.34 ± 0.50	95.1	3.52 ± 0.58	100.2
	48	4.35 ± 0.96	103.0	3.80 ± 0.48	90.0
20%	24	3.49 ± 0.80	99.4	2.68 ± 0.47**	76.3
	48	4.11 ± 1.16	97.3	3.74 ± 0.56	88.6
40%	24	3.18 ± 0.85	90.5	2.51 ± 0.54*	71.5
	48	4.03 ± 1.20	95.4	3.56 ± 0.51**	84.3
60%	24	3.01 ± 0.51	85.7	2.84 ± 0.61	80.9
	48	3.79 ± 1.23	89.8	3.26 ± 0.29	77.2
80%	24	2.59 ± 1.06	73.7	2.47 ± 0.43	70.3
	48	3.80 ± 1.12	90.0	3.03 ± 0.31*	71.8
100%	24	2.25 ± 0.78	64.1	2.28 ± 0.33	64.9
	48	2.94 ± 1.48	69.6	2.64 ± 0.36	62.5

The level of significance was determined by Student's *t*-test: *$p \leq 0.05$, **$p \leq 0.01$.

comparable results in terms of genotoxicity in plant and animal systems. Hence, *A. cepa* bioassay may prove to be a useful alternative for animal experimentation for analyzing cytotoxicity and genotoxicity.

A decrease in MI in *A. cepa* has been previously related to the exposure of pesticides, heavy metals or other chemical pollutants [17,31]. Fiskesjo [14] also reported a significant decline in MI due to the effect of toxic chemicals on spindle apparatus. The MI is contemplated to be reliable for identification of cytotoxic pollutants present in the environment.

3.3.1. MI

MI was calculated by the total number of dividing cells in the cell cycle. The MI of the negative control is 26.1 ± 1.6 after 24 h of exposure and 26.1 ± 1.3 after 48 h. These figures were similar to the results of negative control (distilled water) studied by other researchers; but in some studies the MI for control (distilled water) is even higher than these values [32]. The MI was reduced to 10.3 ± 0.9 and 9.7 ± 0.6 after 24 and 48 h of exposure in case of 100% PTS while in 100% VTS the MI was found to be 24.4 ± 1.7 and 25.4 ± 0.8 after 24 and 48 h respectively. This is the first clear indication of soil enriched with vermicompost being a better alternative. The MI is significantly higher in VTS than control. A concentration dependent inhibition of MI was observed with increasing concentration of PTS. MI observed after exposure of 10% VTS (26.0 ± 0.1) after 48 h was similar as that of control (26.1 ± 1.3). The lowest concentration (10%) of PTS produced minimum negative impact and highest concentration (100%) produced maximum negative impact on mitotic activity of meristematic cells of roots of *A. cepa* (Table 2).

The reduction in MI was dose and duration dependent. The MI in case of VTS is consistent and in PTS it subsequently decreased. The MI in both PTS and VTS was significantly different for all the concentrations at both 24 and 48 h of exposure except 10% at 24 h. This suggests a highly cytotoxic behavior of pesticides. In our studies, we have found a high negative correlation between MI and concentration ($r = -0.953$, $n = 6$, $P < 0.05$) after 24 h and ($r = -0.947$, $n = 6$, $P < 0.05$) after 48 h of treatment for extracts of PTS while MI from VTS extracts also showed a weak correlation with concentration ($r = 0.083$, $n = 6$, $P < 0.05$) after 24 h and ($r = 0.082$, $n = 6$, $P < 0.05$) after 48 h of treatment. The linear relationship between concentrations (10, 20, 40, 60, 80, 100%) and MI of PTS and VTS was obtained by regression analysis (Fig. 1). On the other hand the addition of vermicompost plays an important role in promotion of cell division and proliferation. The MI for VTS can be related to previous reports that show an increase in plant height, number of leaves, fruit weight in vermicompost treated field than control. For the evaluation of cytotoxic and toxic potential of contaminants for environmental pollution monitoring, the reduction or increase in MI is a principal indicator [33]. Vermicompost is known to provide impetus to growth of various plant species like strawberry, groundnut, chilli, garlic, tomato, sweetcorn.

3.3.2. CA

The occurrence of chromosomal abnormalities were more prominent in 100% concentration of PTS, both after 24 and 48 h of exposure with mild frequency of aberrations observed after 24 and 48 h of exposure in VTS (Fig. 2). The major CA was noted as stickiness, laggards, C-mitosis, vagrants, fragments, chromosomal bridges (Fig. 3). The lowest frequency of CA was observed to be 2% in control after 24 h and in 10% VTS after 24 h. The highest frequency of CA (54.5%) was found to be in 100% of PTS extract after 48 h (Tables 3 and 4). PTS significantly ($P < 0.05$) induced higher CA than VTS. The percentage of CA showed positive correlation with concentration of extract after 24 h of treatment in PTS extract ($r = 0.989$, $n = 6$, $P < 0.05$) and after 48 h of treatment in PTS extract ($r = 0.931$, $n = 6$, $P < 0.05$); also after 24 h ($r = 0.945$, $n = 6$, $P < 0.05$)

Table 2
MI and CA (mean ± standard deviation) of the root meristem cells of *A. cepa* exposed to various concentrations of VTS and PTS extracts after 24 and 48 h.

Concentration	Exposure time (h)	MI[a]		CA[b]	
		VTS	PTS	VTS	PTS
Control	24	26.1 ± 1.6	26.1 ± 1.5	2.0	2.0
	48	26.1 ± 1.3	26.0 ± 1.3	2.7	4.5
10%	24	24.5 ± 2.2	24.1 ± 0.2	2.0	3.7
	48	26.0 ± 0.1	21.9 ± 0.7***	3.2	15.2
20%	24	25.1 ± 1.8	19.5 ± 1.3**	2.5	10.0
	48	24.2 ± 0.9	19.7 ± 0.5***	3.5	31.5
40%	24	27.2 ± 3.3	16.6 ± 0.5***	3.5	19.7
	48	27.1 ± 1.3	15.1 ± 0.6***	4.7	33.0
60%	24	28.1 ± 0.5	12.7 ± 0.5***	4.7	24.0
	48	26.4 ± 0.4	10.8 ± 0.3***	5.2	34.5
80%	24	26.2 ± 0.5	12.2 ± 0.3***	6.2	31.5
	48	25.9 ± 0.9	9.5 ± 1.0***	6.2	47.2
100%	24	24.4 ± 1.7	10.3 ± 0.9***	5.5	36.5
	48	25.4 ± 0.8	9.7 ± 0.6***	6.7	54.5

The level of significance was determined by Student's *t*-test: **$p \leq 0.01$, ***$p \leq 0.001$.
[a] Out of 4000–6000 cells examined for MI.
[b] Out of 1000 cells examined for CA.

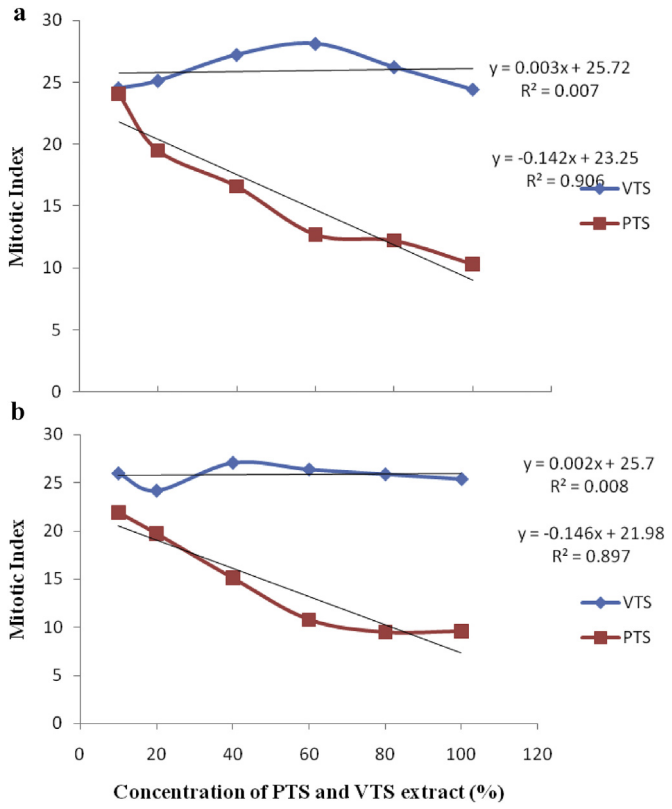

Fig. 1. Relationship between different concentrations of PTS and VTS extract and MI after (a) 24 h and (b) 48 h of treatment in *A. cepa* root chromosomal aberration assay.

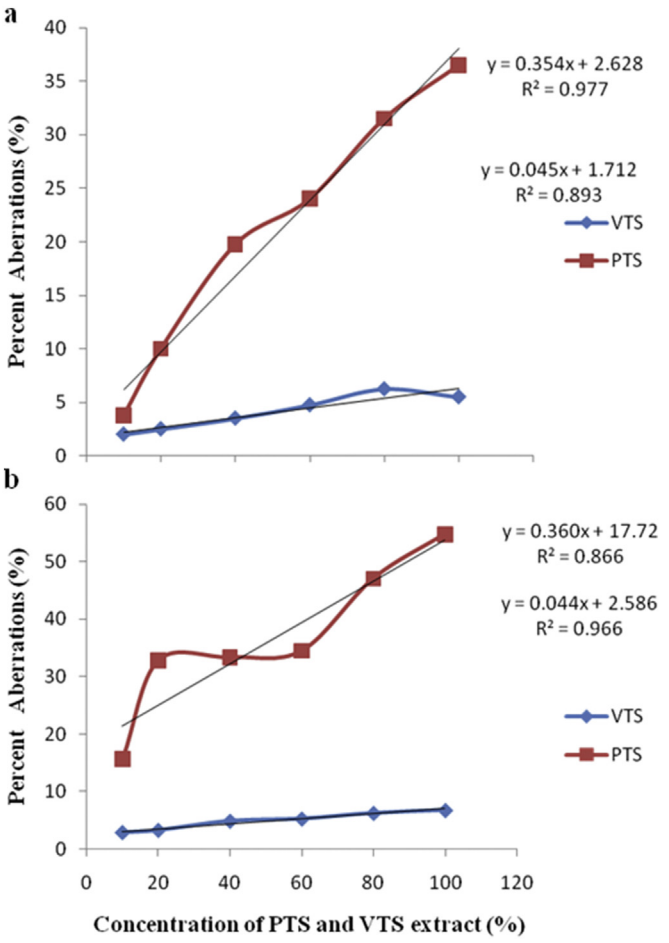

Fig. 2. Relationship between different concentrations of PTS and VTS extract and percentage aberration after (a) 24 h and (b) 48 h of treatment in *A. cepa* root chromosomal aberration assay.

and 48 h ($r = 0.983$, $n = 6$, $P < 0.05$) of treatment with VTS extract. Thus, the rate of CA increased as the concentration of extracts increased. Chromatid breaks give rise to bridges and fragments. The formation of bridges may also be ascribed to unequal exchanges that lead to formation of dicentric chromosomes which are equally pulled at both poles in anaphase stage [34]. The breakage and fusion of chromosomes and chromatids or changing activation of replication enzymes are also responsible for formation of bridges [35]. Spindle anomalies lead to vagrant chromosomes. The failure of chromosomes or acentric fragments to move to either pole is responsible for formation of laggards [36]. The unequal translocation, inversions of chromosome segment and the formation of chromatin bridges attribute to stickiness results in non-separation of chromosomes at anaphase [36]. Entanglement of inter chromosomal chromatin fibers or affected peripheral proteins such as DNA topoisomerase II also leads to stickiness. C-mitosis occurs when a cell or its progeny becomes polyploid [37]. The term 'c-mitosis' was coined by Levan [38] so as to depict the effect of a chemical which prevents spindle microtubule assembly by dissociation of disulphide bonds the way colchicine acts. Stickiness is an irreversible chromosome abnormality that leads to cell death. Chemicals can produce genotoxic effects either directly or indirectly by inhibiting the DNA repair system; for example by competing with certain ions essential for DNA polymerases [39]. The aberrations induced by direct genotoxic effects may be repaired by intervention of DNA repair mechanisms to maintain the integrity of the genome [40]. But indirect genotoxic effects prove to be exponentially toxic. Glyphosate is one such pesticide which decreases DNA repair [41]. In the present study, anaphase lags were found to be more in PTS treatment for both 24 h (44%) and 48 h (28%) of exposure out of total aberrations. This was followed by C-mitosis in both 48 h (23%) and 24 h (18%). Anaphase lags show two groups of anaphasic chromosomes that lay close to each other near the equatorial plate. With respect to CGA in PTS the chromosomal breaks in 24 h (10%) and 48 h (10%) was more than chromosomal bridges.

In case of VTS, out of total aberrations, chromatin breaks were found to be maximum in 24 h (21.6%) and 48 h (20.8%) followed by C-mitosis in 24 h (20.1%). Vagrants were found to be the least (9.9%). The marked number of CA in PTS depicts the genotoxic effects of pesticides on *A. cepa*. These results are in accordance to reports describing the vegetable extracts showing CA in *A. cepa* because of pesticide residues [42] and also with some previous studies that report various chromosomal abnormalities like vagrant chromosomes, chromosomal fragments at anaphase and telophase and multipolar anaphases depicting genotoxicity of agricultural soil [43]. Leme et al. [44] also reported various chromosomal abnormalities while assessing the genotoxicity of a soil matrix contaminated with bio-diesel. Similar studies from other parts of the world reported various mitotic and chromosomal abnormalities predicting the genotoxic and clastogenic potential of contaminated soil [45]. The higher MI and lower frequency of CA in the samples from VTS compared with PTS are indicative of a decrease in the soil genotoxicity. However, the percentage of CA for cells exposed to VTS extracts slightly increase with the concentration. This could be ascertained to the fact that effects of vermicompost on plant growth and yield depend largely upon production method, species of earthworm, storage and cultivation conditions and also on

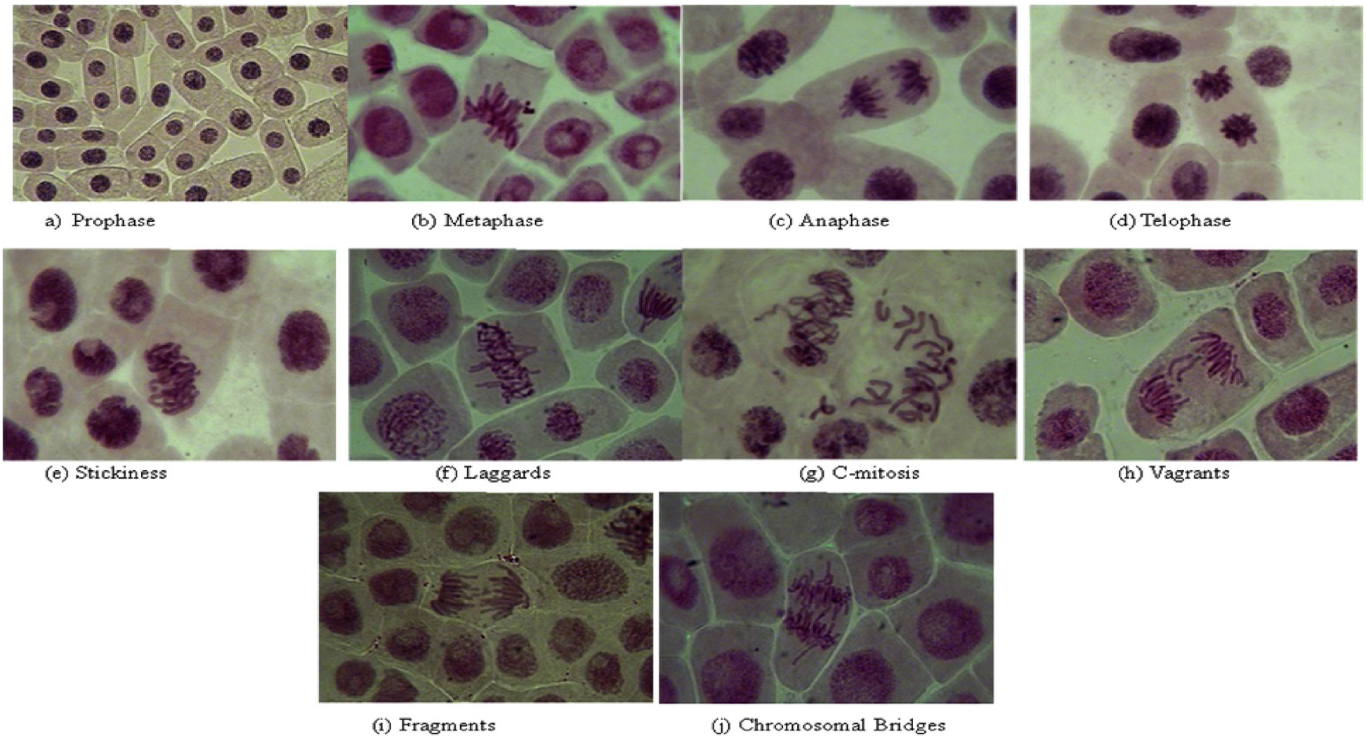

Fig. 3. Root tip cells of *A. cepa* showing normal stages of mitosis (a–d) and chromosomal anomalies (e–j).

genotype of plant [46,47]. This may be a possible reason for the slight rise in toxicity (increase in CA) in VTS extracts. The presence of agrochemicals or other pollutants that have reached and persisted in the organic fields through irrigation or nearby pollutant sites can also be asserted as another possible reason for the slight increase in CA in VTS. Dragoeva et al. [48] also reported higher MI and low CA in the soil samples from the field in conversion period for excluding the use of agrochemicals when compared with the field under conventional agriculture utilizing appropriate agrochemicals. However, vermicompost delineates positive results based on a number of agricultural studies. Gopinath et al. [49] reported an improvement in water and air availability, encouraging root growth and seedling emergence when vermicompost was applied @ 6×10^{-3} kg m^{-2} for two consecutive growing seasons. In case of tomato, vermicompost amended soil pots (2 kg m^{-2}) showed better growth than plants grown in inorganic fertilizer

Table 3
Different CA (physiological and clastogenic) in the root meristem cells of *A. cepa* exposed to various concentrations of PTS extract for 24 and 48 h.

Type of CA	Time of exposure (h)	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]
		Control	10%	20%	40%	60%	80%	100%
PA								
C-mitosis	24	–	–	20	27	42	58	80
	48	15	28	75	82	80	115	105
Anaphase lag	24	7	15	25	115	123	145	135
	48	5	57	95	97	85	135	135
Stickiness	24	–	5	22	20	17	30	42
	48	10	20	70	65	70	67	100
Vagrants	24	5	5	13	15	28	42	50
	48	–	15	40	53	47	60	112
Total PA	24	12	25	80	177	210	275	307
	48	30	120	280	297	282	377	452
CGA								
Chromosomal bridges	24	3	–	5	3	10	13	25
	48	–	10	15	16	30	33	40
Chromosomal breaks/ fragments	24	5	12	15	17	20	27	33
	48	15	22	20	17	33	62	53
Total CGA	24	8	12	20	20	30	40	58
	48	15	32	35	33	63	95	93
Total aberration (PA + CGA)	24	20	37	100	197	240	315	365
	48	45	152	315	330	345	472	545
Percent aberration	24	2	3.7	10	19.7	24	31.5	36.5
	48	4.5	15.2	31.5	33	34.5	47.2	54.5

[a] Out of 1000 cells examined.

Table 4
Different CA (physiological and clastogenic) in the root meristem cells of *A. cepa* exposed to various concentrations of VTS extract for 24 and 48 h.

Type of CA	Time of exposur (h)	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]	No. of aberrant cells[a]
		Control	10%	20%	40%	60%	80%	100%
PA								
C-mitosis	24	–	5	5	5	7	17	14
	48	5	8	5	10	10	13	10
Anaphase lag	24	7	5	5	7	5	8	5
	48	5	5	7	8	7	10	7
Stickiness	24	–	5	3	5	10	12	10
	48	5	4	5	7	10	5	7
Vagrants	24	5	–	–	3	8	8	8
	48	–	–	3	5	3	7	8
Total PA	24	12	15	12	20	30	45	37
	48	15	17	20	30	30	35	32
CGA								
Chromosomal bridges	24	3	5	8	5	7	7	13
	48	10	10	5	7	10	12	10
Chromosomal breaks/ fragments	24	5	–	5	10	10	10	17
	48	2	5	10	10	12	15	13
Total CGA	24	8	5	5	15	17	17	30
	48	12	15	15	17	22	27	35
Total aberration (PA + CGA)	24	20	20	25	35	47	62	55
	48	27	32	35	47	52	62	67
Percent aberration	24	2	2	2.5	3.5	4.7	6.2	5.5
	48	2.7	3.2	3.5	4.7	5.2	6.2	6.7

[a] Out of 1000 cells examined.

amended soil [50]. A significant increase in the growth and productivity was found in strawberries cultivated with 0.5 and 0.75 kg m^{-2} of vermicompost in comparison to strawberries cultivated with equivalent doses of mineral fertilizers [47]. However, in this study a comparison between the volume of pesticide taken and vermicompost taken seems irrelevant. This could be attributed to the reason that their mode of action is entirely different and using a similar volume of both will lead to ineffectiveness or negative impact of either of them. The present study indicated the genotoxic potential of PTS extract and also an inclination towards the practicability of vermicompost to ameliorate the toxicity/genotoxicity. Thus, it can be concluded that vermicompost might be overall beneficial in terms of soil health, plant health and crop productivity ensuring safe standards for the biodiversity that help maintain the eco-equilibrium. *A. cepa* test might be used for monitoring of genotoxic pollution of the soils without preliminary extraction of the chemicals they contain [48].

4. Conclusions

A. cepa test is a useful bio indicator of cytotoxicity and genotoxicity and serves as an alert for the population that uses pesticides indiscriminately. The genotoxicity study of soil samples depicts that the use of both inorganic and organic pesticides leads to soil pollution and contamination. Also, the direct use of industrial waste and wastewater, sewage sludge in agricultural land contains much more toxic elements along with useful nutrients. This provides us an insight towards the better understanding of soil contaminants to dodge the potential risks associated with contaminated agricultural soils and affiliated food chains. Pesticides and other pollutants are cytotoxic and have the ability to damage DNA and thus also show genotoxic effects. Vermicompost on the other hand is non-cytotoxic in nature and helps in efficient cell growth. Also, the physico-chemical analysis reveal lower TOC, EC and higher pH and more nutrient content in VTS in comparison to PTS. By the use of vermicompost in lieu of pesticides, the biodiversity and useful organisms do not perish which further tends to elevate the physical and nutritional status of crops. However, the calibration for inorganic fertilizers and plant breeding for agricultural benefits is largely carried out in order to provide maximum yields. Such detailed knowledge and interactions between organic fertilizers and plants still need to be substantiated in order to gain consumer confidence in this organic fertilizer. Further, a clearer concept for vermicompost is required to comprehend its variable and multiplex structure which can exert multifarious effects on soil-functioning. The assessment by *A. cepa* suggests that in several respects usage of vermicompost is far much better than the pesticides.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.serj.2018.01.005.

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