Genotoxicity of *Thermopsis turcica* on *Allium cepa* L. roots revealed by alkaline comet and random amplified polymorphic DNA assays

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Abstract This study was undertaken to evaluate genotoxic potential of *Thermopsis turcica* aqueous extracts on the roots of onion bulb (*Allium cepa* L.) by comet assay and random amplified polymorphic DNA technique. The *Allium* root growth inhibition test indicated that the EC_{50} and $2 \times EC_{50}$ values were 8 and 16 mg/ml concentrations of *T. turcica* aqueous extracts, respectively. The negative control (distilled water), positive control (methyl methane sulfonate, 10 mg/l) and 8 and 16 mg/ml concentrations of *T. turcica* extracts were introduced to the roots of onion bulbs for 24 and 96 h. The root growth, DNA damage in root cells and randomly amplified polymorphic DNA (RAPD) profiles of root tissue were used as endpoints of the genotoxicity. The comet assay clearly indicated that dose-dependent single strand DNA breaks in the root nuclei of onions were determined for the treatment concentrations of *T. turcica* extracts. In comparison to RAPD profile of negative control group, RAPD polymorphisms became evident as disappearance and/or appearance of RAPD bands in

treated roots. The diagnostic and phenetic numerical analyses of RAPD profiles obviously indicated dose-dependent genotoxicity induced by *Thermopsis* extracts. In conclusion, the results clearly indicated that water extract of *T. turcica* has genotoxic potential on the roots of onion bulbs as shown by comet assay and RAPD technique.

Keywords Plant extract · DNA damage · Comet assay · RAPD

Introduction

Thermopsis turcica Kit Tan, Vural and Küçüködük is distributed in a narrow area around Eber and Akşehir Lakes of Turkey (Tan et al. 1983). This native and endemic plant is potentially an important genetic resource for Turkey as it holds an unusual morphological character to produce 3–4 free pods from a single flower (Davis et al. 1988). This is a unique character among 12,000 papilionoid species including economically and ergonomically important legume species such as pea, soybean and common bean (Tucker 2003). In-situ and ex-situ efforts have been in progress to conserve this critically endangered plant species from extinction (Cenkci et al. 2007, 2008, 2009a). Certain species of the legume genera such as *Baptisia*, *Cytisus*, *Laburnum*, *Lupinus*, *Sophora* and *Thermopsis* are rich in lupine alkaloids and flavonoids,

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and have been incriminated as plants poisonous to livestock. Among them, *Thermopsis* R.Br. is reported as rich in anagyrine, thermopsine, termopsidine, 5,6-dehydrolupanine, cytisine, lupanin, and N-methylcytisine (Ohmiya et al. 1974; Dement and Mabry 1975; Saito et al. 1989).

The medicinal plants have been traditionally consumed overall in the world without evidence in the literature for safety of their consumptions. The composition of these natural extracts that apparently exhibit only beneficial properties may include chemical components with mutagenic, teratogenic and/or carcinogenic activities (Alves dos Santos et al. 2008). In recent years, genotoxic and mutagenic effects of medicinal plants have been screened by using molecular tools such as cytogenetic assays (Portmann et al. 2012; Şekeroğlu and Şekeroğlu 2012), comet assay (Kalantari et al. 2012; Nitzsche et al. 2013), Ames test (Santos et al. 2006; Hong and Lyu 2011) and oxidative stress assays (Skandrani et al. 2010).

For monitoring genotoxicity, it is important to use sensitive but nonspecific assays that indicate a wide range of DNA damage types. The alkaline comet assay or single cell gel electrophoresis has been widely used to evaluate in vitro and/or in vivo genotoxicity of suspicious chemicals and provides a direct determination of the single and double-stranded DNA breaks in the response of individual cells (Singh et al. 1988; Cigerci et al. 2013; Khallef et al. 2013). The random amplified polymorphic DNA (RAPD) technique has been also used to show mutagenic effects of genotoxins such as heavy metals, pesticides and UV radiation (Atienzar et al. 1999, 2000; Ceneci et al. 2009a, b, 2010a, b). On the other hand, there is no published report describing the utilization of RAPD assay to show genotoxic potential of plant extracts on plant systems such as *Allium cepa*. The present study was aimed to evaluate the potential in vitro mutagenic effects of *T. turcica* extracts on *A. cepa* roots by the Comet and RAPD assays.

Materials and methods

Plant material

T. turcica specimens were collected from a small plant population located in a marsh area (ca. 960 m altitude) at the south of Eber Lake, Turkey, in 2011. The leaf

samples were immediately dried after collection in a circulating air oven at 65–70 °C for 72 h. The oven-dried leaves were later milled to a fine powder by using a kitchen blender. 10 g powdered leaf material of *T. turcica* was extracted with 100 ml hot-water in a Soxhlet apparatus. After filtering the extract through a filter paper to remove particulates, the stock solution (100 mg/ml) was diluted with distilled dH₂O to eight different concentrations (1, 5, 10, 15, 20, 40, 60, 80 and 100 mg/ml). The stock and study solutions were kept in a refrigerator at 4 °C until use.

Growth inhibition assay and experimental condition with *A. cepa* L.

The bulbs (3.5–4.5 cm in diameter) of common onion (*A. cepa* L., 2n = 16) were used in growth inhibition assay as a 96 h semi-static exposure test (Rank 2003). The yellow shallows and dry bottom plate inside the root primordial of onion bulbs were carefully peeled off prior to assays. The EC₅₀ of *T. turcica* water extracts were determined with a similar protocol as described by Çelik and Aslantürk (2010). For each extract sample, a series of five bulbs were placed in tap water (pH 7.3) for 48 h. Subsequently, the onion bulbs were treated with 1, 5, 10, 20, 40, 60, 80 and 100 mg/ml concentrations of *T. turcica* extracts, and were kept at climatic test room at 25 ± 1 °C in dark for 96 h. Randomly selected ten roots of each onion bulbs (n = 5) were measured to determine EC₅₀ of *T. turcica* extracts.

For the study experiments, well rooted bulbs were washed under running tap water for 5–10 min and then subjected to treatments. Tap water was used as negative control (NC) and 10 ppm methyl methane-sulfonate (MMS) is used for the positive control (PC). 8 mg/ml (EC₅₀) and 16 mg/ml (2×EC₅₀) concentrations of *T. turcica* extracts were used as experimental groups. The root tips of treated onion bulbs were harvested at 24 and 96 h experiment initiation in order to use them in two different genotoxicity assays as described below (Yıldız et al. 2009).

Comet assay

20–30 seedlings were placed in a petri dish kept on ice and spread with 500 µl of ice-cold Tris–MgCl₂ buffer (0.2 M Tris, pH 7.5; 4 mM MgCl₂–6H₂O; 0.5 % w/v Triton X-100). The roots were immediately chopped

with a fresh razor blade and isolated root nuclei collected in the buffer. Each microscope slide was pre-coated with a layer of 1 % NMPA and thoroughly dried at room temperature. Then, 100 μ l of 0.8 % LMPA at 37 °C was mixed with 20 μ l of the nuclear suspension and dropped on top of the first layer. The slides were allowed to solidify for 5 min on an ice-cooled tray and were then immersed in ice-cold lysing solution (1 M NaCl; 30 mM NaOH, 0.5 % w/v SDS, pH 12.3) for 1 h. Subsequent to lysing, the slides were placed in a horizontal gel electrophoresis chamber and the DNA was allowed to unwind for 1 h in the electrophoretic buffer, containing 30 mM NaOH and 1.5 mM EDTA at pH 12.3. Electrophoresis was then conducted for 20 min at 25 V (1 V cm^{-1}) in the chamber cooled on ice. Following electrophoresis, the slides were rinsed three times with cold distilled water for neutralization and stained with 60 μ l EtBr (20 $\mu\text{g ml}^{-1}$) and covered with a cover slip (Rucinska et al. 1999). 100 comets (100 comets/slide) were scored visually as belonging to one of five classes (0—undamaged, 1—mild damage, 2—moderate damage, 3—severe damage, 4—complete damage) using a fluorescence microscope (Collins 2004). Thus, the total score for 100 comets could range from 0 (all undamaged) to 400 (all damaged). Three slides were evaluated for treatment and each treatment was repeated at least twice. Arbitrary Unit used to express the extent of DNA damage was calculated as follows Eq. 1):

$$\text{Arbitrary Unit(AU)} = \sum_{i=0}^4 N_i x_i \quad (1)$$

where N_i number of cells in i degree, i degree of damage (0, 1, 2, 3, 4).

Genomic DNA isolation

The genomic DNA was extracted as described by Doyle and Doyle (1987) with some modifications. In summary, 0.25 g root tissue was ground to a fine powder in liquid N_2 and mixed with 1 ml CTAB buffer [2 % CTAB, 1.4 M NaCl, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1 % PVP-40, and 0.2 % 2-mercaptoethanol] and 0.4 ml chloroform. The mixture was incubated at 60 °C for 30 min with occasional shaking and with leaving the gas pressure occurring in the tubes. After centrifugation at 12,000 rpm, the aqueous phase

was transferred into a new tube and digested with RNase A at 60 °C for 10 min. The DNA was precipitated by adding isopropanol (60 % of final volume) and subsequently by incubating at 4 °C for 30 min. The pellet was collected at room temperature by a brief centrifugation. The pellet was washed (with 70 % ethanol), air-dried and finally dissolved in TE buffer. The concentration and purity of isolated genomic DNA was spectrophotometrically determined. The DNA stocks were stored at –20 °C until use.

RAPD procedures

PCR reactions were performed in reaction mixtures of 25 μ l containing: 25 ng of genomic DNA, 1.7 μ M 10-mers primer (Operon Technologies, Inc., Alameda, CA, USA), 50 μ M of each dNTPs 10 $\times$ PCR reaction buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl, 15 mM MgCl_2 , 0.8 % Nonidet P40) and 1U *Taq* DNA polymerase (Fermentas, Hanover, MD, USA). An initial screening of 40 RAPD decamer primers was performed in order to test amplification profiles for polymorphism, readability and reproducibility. Sequences (5' $\rightarrow$ 3') of 13 primers successfully utilized were: CAG GCC CTT C (OPA-01); TGC CGA GCT G (OPA-02); AAT CGG GCT G (OPA-04); TCT GTG CTG G (OPA-14) GAC CGC TTG T (OPA-17); GTT GCG ATC C (OPA-20); GTT TCG CTC C (OPB-01); CATCCCCCTG (OPB-03); GGA CTG GAG T (OPB-04); TGG GGG ACT C (OPB-09); CCT TGA CGC A (OPB-12); TTC CCC CGC T (OPB-13); TCC GCT CTG G (OPB-14), respectively. A thermocycler was programmed for 4 min at 94 °C, 40 consecutive cycles each consisting of 45 s at 94 °C, 45 s at 36 °C, 60 s at 72 °C, and followed by 8 min at 72 °C. Negative controls with bidistilled water replacing the template DNA were always included to monitor for contamination. RAPD-PCR products and GeneRuler 100 bp DNA Ladder Plus (Fermentas) were separated in 1.8 % agarose gel under 1 $\times$ TBE buffered electrophoresis. DNA bands were stained with ethidium bromide, visualized and photographed under UV light. The size of each amplification product was automatically estimated using the UVIssoft image analyzer system. For accuracy in comparisons of the results, PCR products of the treated and untreated roots were run on the same gel. All reactions were performed twice in order to verify the reproducibility of all polymorphic bands in laboratory.

RAPD profiles and data analysis

Only reproducible and clear amplification bands were scored for the construction of the data matrix. The obvious changes observed in RAPD profiles (disappearance and/or appearance of bands in comparison with untreated control treatments) were evaluated (Atienzar et al. 1999; Cenkci et al. 2009b). Amplified products were analyzed according to Lynch and Milligan (1994). A pairwise matrix of distances among the untreated and treated plant samples was constructed for RAPD (13 primers) data using Jaccard's algorithm. The distance data were used to generate Neighbor-Joining (NJ) trees after 1,000 replicate bootstrap tests for all experimental groups. The dendrogram was generated from the matrix according to Saitou and Nei (1987) using the FreeTree (Pavlicek et al. 1999) and TreeView (Page 1996) software packages. The genomic template stability (GTS %) was calculated for each primer by the following formula: $GTS (\%) = (1 - a/n) \times 100$, as where a is the average number of changes in DNA profiles and n the number of bands selected in control DNA profiles (Atienzar et al. 2000). The average was then calculated for each experiment group exposed to different time and concentrations to *T. turcica* extracts.

Statistical analysis

Data on root growth and Comet assay are mean values of three independent replicates. Significant differences among treatments were analyzed by one-way ANOVA, taking $P < 0.05$ as significant level using SPSS v15.0 for Windows software.

Results and discussion

The EC_{50} and $2 \times EC_{50}$ concentrations for *T. turcica* extracts were determined as 8 and 16 mg/ml, respectively, in the root growth inhibition assays. The treatment with *T. turcica* extracts severely stunted (≥ 20 mg/ml) or significantly inhibited (< 20 mg/ml) the root growth of onion bulbs in comparison to the untreated control group. At least two-fold decrease in the root growth was reported in similar studies conducted with lower (10 mg/ml) concentrations of herbal extracts (Çelik and Aslantürk 2010;

Rathnasamy et al. 2013; Macêdo et al. 2008; Akinboro and Bakare 2007). The appearance of stunted roots and inhibition of rooting are indicative of different biological end-points such as chromosomal aberrations, mutations and apoptosis (Cenkci et al. 2010a).

The mild alkaline comet assay (pH 12.3) allows the detection of single- and double-strand breaks, incomplete excision-repair sites and cross-links. It has been used to screen genotoxic effects of environmental pollutants in the root nuclei of various plant species (Gichner et al. 2006). In the present study, DNA damage values for the negative control at 24 and 96 h were 94 and 90 AU, respectively (Table 1). The highest genotoxic activity was observed in the positive control at 24 h (230 AU) and 96 h (270 AU). In comparison to the negative control, the exposure of *T. turcica* extracts significantly ($P \leq 0.05$) induced DNA damage in the root nuclei of onion bulbs at both concentrations (8 and 16 mg/ml) and at both exposure times (24 and 96 h). There was no significant difference in the genotoxic activity between 8 and 16 mg/ml treatment groups after 24 h exposure. 16 mg/ml *Thermopsis* extract and the positive control has similar genotoxic activity on the root nuclei at 96 h. The comet assay results of the present study clearly indicated that *T. turcica* extract has mutagenic effects on the root nuclei of onion bulbs in a dose- and time-dependent manner. The genotoxic activity of plants extracts were successfully shown on different biological systems such as HTC and V79 cells (Maistro et al. 2004), the HepG2 cell line (Lamy et al. 2008), human

Table 1 The comet assay score in the roots of onion bulb (*A. cepa* L.) exposed to negative control (NC), positive control and two dilutions (8 and 16 mg/ml) of *T. turcica* water extracts for 24 and 96 h

Treatment	DNA damage scores (mean $\pm$ SD)	
	24 h	96 h
Negative control (dH ₂ O)	94 $\pm$ 4.00c	90 $\pm$ 1.55c
Positive control (10 ppm MMS)	230 $\pm$ 13.25a	270 $\pm$ 6.41a
8 mg/ml of <i>T. turcica</i> extract	161 $\pm$ 1.50b	210 $\pm$ 12.50b
16 mg/ml <i>T. turcica</i> extract	182 $\pm$ 2.05b	250 $\pm$ 6.12a

The values are given as means of nine replicates. The values marked by the same letter are not significantly different at $P < 0.05$ level based on Duncan's multiple range analysis

lymphocytes (Basaran et al. 1996) and *A. cepa* as presented in this study by using comet assay.

It is suggested that after proper optimization, the RAPD as a reliable, sensitive and reproducible assay, has the potential to detect DNA damage (e.g. DNA adducts, DNA breakage) and mutations (point mutations and large rearrangements) and therefore can be applied to genotoxicity studies (Atienzar and Jha 2006). In this study, the forty 10-mer oligo nucleotide primers of 50–70 % GC content utilized for screening the onion genome for changes. However, only 13 primers among them amplified reproducible and informative RAPD profiles. A total of 159 different DNA fragments was ranging between 158 (OPA-01) and 3,300 bp (OPA-01) with these primers. From those different DNA fragments, 119 of them (75 %) were monomorphic and the remains (25 %) were polymorphic DNA bands. The RAPD profiles showed major differences among the negative control, positive control and *T. turcica* treated onion roots with apparent changes (disappearing of a normal band

and/or appearing of an extra band) in the number and size of amplified DNA fragments. The selected RAPD profiles of four informative primers (OPB-04, OPB-09, OPB-12 and OPB-13) are shown in Fig. 1. Table 2 summarizes these alterations detected in RAPD profiles of treated onion bulbs. The total number of appeared and disappeared RAPD bands was six for positive control, 6 for 8 mg/ml treatment and nine for 16 mg/ml treatment after 24 exposure time (Table 2). After 96 h treatment, on the other hand, total number of appeared and disappeared RAPD bands was 15 for positive control, 19 for 8 mg/ml treatment and 20 for 16 mg/ml treatment (Table 2). In summary, the total number of band changes was increased with an increase in exposure time and the concentration of the water extract of *T. turcica*. In the present study, we have evaluated the potential of the RAPD assay to measure *T. turcica* extracts-induced DNA effects in the roots of onion bulbs: disappearance of normal RAPD bands and appearance of new PCR products occurred in the profiles. The disappearance of the

Fig. 1 RAPD profiles generated using primers OPB-04, OPB-09, OPB-12 and OPB-13 for genomic DNAs for the root tips of onion bulb (*A. cepa* L.) exposed to the negative control (NC), positive control (PC; MMS), and two dilutions (8 and 16 mg/ml) of *T. turcica* extracts for 24 and 96 h. M: Gene Ruler 100 bp Plus DNA Ladder (100–3,000 bp)

Table 2 RAPD amplifications showing the number of bands (appearance and disappearance, A&D) with molecular size (bp) in 8 and 16 mg/ml *T. turcica* extracts exposed root tips ofonion bulb (*A. cepa* L.) for 24 and 96 h using multiple primers contrasted with negative (NC) and positive controls (PC; methyl methanesulfonate, MMS)

Primers	NC	24 h			96 h		
		PC	8 mg/ml	16 mg/ml	PC	8 mg/ml	16 mg/ml
OPA-01	12	A	–	620	620	–	620
		D	–	–	–	–	–
OPA-02	7	A	–	–	294; 863	–	294
		D	–	–	–	–	–
OPA-04	8	A	–	289	163	744	289; 645
		D	–	–	–	–	–
OPA-14	5	A	–	–	916; 469	–	916
		D	–	–	–	–	–
OPA-17	18	A	262	–	–	262	262; 1,092; 1,229
		D	–	–	–	204; 1,412; 1,528	204; 1,412; 1,528
OPA-20	20	A	480	–	1,331	–	–
		D	–	–	–	729; 800; 1,084; 1,700	399; 466; 1,700
OPB-01	15	A	–	–	–	–	710
		D	–	–	–	–	2,447
OPB-04	12	A	–	–	–	824	874
		D	–	–	–	–	–
OPB-09	9	A	520	–	–	520	520
		D	–	–	–	–	–
OPB-03	12	A	–	–	294; 511	511	511
		D	937; 1,406	–	–	–	–
OPB-12	11	A	–	1,843	1,843	–	1,843
		D	–	–	–	1,403	–
OPB-13	9	A	–	–	–	–	–
		D	1,318	1,181; 1,318	–	–	1,318
OPB-14	8	A	–	788	788	–	788
		D	–	–	917	–	917
Total	146		3 (A):3 (D)	4 (A):2 (D)	9 (A):0 (D)	4 (A):11 (D)	11 (A):8 (D)

bands of the negative control in treatment groups may be related to DNA damage (e.g. single- and double-strand breaks), point mutations and/or complex chromosomal rearrangements induced by genotoxins (Cenkci et al. 2009b, 2010a, b). Appearance of new PCR products (extra bands) was also detected in RAPD profiles may reveal a change in some oligonucleotide priming sites due to mutations [new annealing events, large deletions, and/or homologous recombinations (Atienzar et al. 1999)]. The genomic template stability (GTS %) values, a qualitative measure reflecting changes in RAPD profiles was calculated for each 13 primers (Table 3). In comparison to the negative control, GTS (%) values were decreased

obviously with the treatment of *T. turcica* extracts, either with 8 or 16 mg/ml concentration for 24 h. The extension of exposure time of *T. turcica* water extracts on the onion roots results in an enhanced decrease in GTS values. On the other hand, 8 and 16 mg/ml concentrations of *T. turcica* extracts had similar reluctant effect on GTS values in both 24 and 96 h treatment groups. The results indicated that genomic template stability (GTS) in onion bulbs was significantly affected by *T. turcica* extracts. Genomic template stability is related to the level of DNA damage, the efficiency of DNA repair and replication. Therefore, a high level of DNA damage does not necessarily decrease the genomic template stability (in

Table 3 Genomic template stability (GTS %) in the roots of onion bulbs exposed to negative control (NC), positive control and two dilutions (8 and 16 mg/ml) of *T. turcica* water extracts for 24 and 96 h

Genomic template stability for each 10-mers oligo was estimated by the formula $GTS (\%) = (1 - a/n) \times 100$, where a is the average number of changes in DNA profiles and n the number of bands selected in control DNA profiles

	24 h				96 h			
	NC	PC	8 mg/ml	16 mg/ml	NC	PC	8 mg/ml	16 mg/ml
OPA-01	100.0	83.3	83.3	83.3	100.0	83.3	83.3	83.3
OPA-02	100.0	100.0	85.7	71.4	100.0	100.0	100.0	85.7
OPA-04	100.0	62.5	50.0	87.5	100.0	87.5	50.0	75.0
OPA-14	100.0	100.0	80.0	100.0	100.0	100.0	40.0	80.0
OPA-17	100.0	88.9	94.4	100.0	100.0	38.9	11.1	33.3
OPA-20	100.0	58.3	75.0	91.7	100.0	25.0	41.7	58.3
OPB-01	100.0	100.0	100.0	100.0	100.0	93.3	100.0	86.7
OPB-04	100.0	100.0	100.0	100.0	100.0	91.7	83.3	50.0
OBP-03	100.0	84.6	100.0	100.0	100.0	84.6	92.3	92.3
OPB-09	100.0	88.9	100.0	100.0	100.0	100.0	88.9	88.9
OPB-12	100.0	100.0	90.9	90.9	100.0	100.0	90.9	90.9
OPB-13	100.0	88.9	77.8	100.0	100.0	88.9	88.9	77.8
OPB-14	100.0	87.5	100.0	100.0	100.0	87.5	87.5	75.0
Total	100.0	87.9	87.4	86.5	100.0	83.1	73.9	75.2

comparison to a low level of DNA alterations), because DNA repair and replication are inhibited by the high frequency of DNA damage (Atienzar et al. 2000). The dendrogram was constructed using Neighbor-Joining method in order to determine phenetic relationships among untreated and treated groups. The onion bulbs exposed to negative controls (NC), positive control (PC) and two dilutions (8 and 16 mg/ml) of *T. turcica* water extracts for 24 and

96 h showed four main branches (Fig. 2). The negative controls of 24 and 96 h were grouped together. The second cluster was consisted from PC-24 h and 8 mg/ml-24 h treatments. However, 16 mg/ml-9 h was grouped alone. Finally, all 96 h treatment groups clustered together (Fig. 2). The change in total RAPD bands (both appeared and disappeared) and GTS values, and also the dendrogram constructed based on RAPD data clearly indicated that *T. turcica* extract has

Fig. 2 The dendrogram using the Neighbor-Joining method based on DNA polymorphism among the roots of onion bulb (*A. cepa* L.) exposed to negative control (NC), positive control (PC) and two dilutions (8 and 16 mg/ml) of *T. turcica* water extracts for 24 and 96 h. The dendrogram was generated from the RAPD data using the FreeTree (Pavlicek et al. 1999) and TreeView (Page 1996) software packages

DNA damaging effect on the root nuclei of onion bulbs at dose- and time-dependent manner. The results of alkaline comet and RAPD assays presented in this research are in agreement and obviously support each other. The agreements of alkaline comet and RAPD assays in genotoxicology studies were previously shown by Cenkci et al. (2009a, b).

The total alkaloid and anagryine-type alkaloid contents of *T. turcica* leaf extracts were reported as 1.48 and 0.69 %, respectively (Şener et al. 1992). The antimicrobial and mutagenic effects of *T. turcica* extracts have been shown (Korcan et al. 2009; Liman et al. 2012; Bali et al. 2014). Although it seems like a medicinal herb, the local people did not consume *T. turcica* for medicinal purposes as their counterparts. For example, *T. lanceolata* R.Br. is used to treat wounds and fever, and used for the fortifying the body (Medicinal Plants in Mongolia 2013) and *T. inflanta* Camb. is consumed to cure joint pains and the seeds orally taken with milk or water as a general tonic to improve health (Singh 2012). The seed powder decoction of *T. montana* Nutt. var. *montana*, on the other hand, was used as cough medicine in America in 1950s (Tilley 2012). However, direct consumption of *Thermopsis* leaves or seeds by domestic animals might cause animal losses or have teratogenic effects on the unborn infants (Keeler et al. 1986; Keeler and Baker 1990). Interestingly, skeletal and muscular disorders have reported for the children or puppies fed with milk of *Thermopsis* grazed animals (McGrath-Hill and Vicas 1997).

In conclusion, the results obtained through the comet assay and RAPD technique clearly indicated that water extract of *T. turcica* has genotoxic potential on the roots of onion bulbs. In this way, our study will contribute to agricultural biological control by using herbal compounds as an alternative to synthetic chemicals. In addition to that, potential risk of these compounds will be determined.

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