Repellent and Fumigant Activities of Tanacetum nubigenum Wallich. ex DC Essential Oils against Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae)

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Abstract: The repellent and fumigant toxicity of essential oils of Tanacetum nubigenum Wallich. ex DC collected from three different habitats (Gothin, Burphu and Glacier) of Uttarakhand Himalayas, India named as TNG, TNB and TNM respectively, were investigated against the adults of red flour beetle Tribolium castaneum (Herbst). Among the three samples tested, TNG was found to more potent exhibiting more repellent effect towards the insects and with LC₅₀ values by fumigant bioassay were 13.23 and 8.32 µl per 0.25 L air at 24 and 48 h exposure of insects to the essential oil respectively. In between other two oil samples, TNM was superior in potency showed LC₅₀ value of 14.22 (24 h) & 8.82 µl per 0.25 L air (48 h). During in vivo study all the essential oil samples significantly protected 500 g of wheat grains for 6 months from insect infestation as compared to non fumigated grains and order of efficacy was TNG>TMN>TNB. There were no side effects of essential oils on germination rate of grains (<85%) exposed for 6 months after fumigation. The present study suggests that essential oil of T. nubigenum can be explored as novel natural fumigants for the control of stored product insects.

Key words: Tanacetum nubigenum, fumigant activity, Tribolium castaneum, wheat grains

1 INTRODUCTION

Red flour beetle Tribolium castaneum (Herbst) is considered to be one of the most serious insect pests in grain storage throughout the world. It causes serious loss in weight and quality of the grains during storage. Earlier records revealed that, the annual overall damage caused by this insect pest was 10-40% of total worldwide production²⁻¹². Use of essential oil based botanical insecticides can be an alternative to synthetic pesticides. These plant based insecticides has found to be effective in pest control due to its high selectivity, no harmful effect on non target organisms, low residual toxicity, ecofriendly, renewable and novel mode of action against stored product insects²⁻³⁰. Several authors have described in vitro and in vivo application of several essential oils as repellent, insecticidal, ovicidal and ovipositional agents in the control of T. castaneum²⁻⁶, however, less attention has been given regarding the essential oil of Tanacetum nubigenum for the control of stored product pests.

T. nubigenum belongs to family Asteraceae is a strongly odorous perennial herb found at high altitude of Uttarakhand Himalayas region. Previous studies on this plant mainly focused on its use by human since prehistoric times and are one of the major sources of culinary, vegetable and medicinal plants all over the world²¹. In addition, few researchers investigated the antimicrobial activity of other Tanacetum species²⁻⁸. The place of occurrence of same species in different ecoclimatic regions influences its toxicity. Chemically essential oils comprise of monoterpenes and sesquiterpenes compounds. Higher terpenes may also be present as minor constituents. The most predominant groups are cyclic compounds with saturated or unsaturated hexacyclic or an aromatic system. Bicyclic (1,8-cineole) and acyclic (linalool, citronellal) examples also make the components of essential oils²⁰. However, intraspecific variabili-
2 EXPERIMENTAL PROCEDURES

2.1 Materials

2.1.1 Insect

The adults of test insect *T. castaneum* were obtained from laboratory cultures and reared in a plastic container (15 cm height × 5 cm diameter) containing wheat flour and yeast (10:1, w:w). The cultures were maintained in continuous darkness at 28 ± 2°C and 70 ± 5% RH. Two to three week old adults of *T. castaneum* were used during entire experiments.

2.1.2 Plant materials

The mature aerial parts of *T. nubigenum* were collected from three alpine locations viz., Gothing, Niti valley (Chamoli), Burphu (Pithoragarh) and Milam Glacier (Pithoragarh) of Uttarakhand Himalayas regions, India during the year 2007-2009. The samples from Gothing, Burphu and Glacier were collected at full flowering stage of the plant and were named as TNG, TNB and TNM respectively. The plant specimen was identified by the aid of herbarium records of Botanical Survey of India (BSI), Northern Circle, Dehradun as well as available literature. Voucher specimens of plant species have been deposited at the Herbarium of BSI, Dehradun with accession numbers BSD112207 (TNG), BSD112210 (TNB) and BSD112211 (TMN).

2.2 Isolation of essential oil

Three hundred grams of shade dried aerial parts of all the samples were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h in order to obtain essential oil. Anhydrous sodium sulphate was used to remove water from essential oil after extraction. Isolated oil of each sample was stored separately in a clean glass vial (20 mL) in a refrigerator at 4 ± 1°C. The oils obtained were dried over anhydrous sodium sulfate.

2.3 GC and GC-MS analysis

Chemical composition of each oil sample was determined by analytical GC-FID and GC-MS technique. The essential oils were analyzed using Agilent 6890N GC (USA), coupled with Flame Ionization Detector (FID). The analysis was carried out using HP-5 (30 m × 0.32 mm; 0.25 µm film thickness; Thermo fisher Scientific; 5% Diphenyl/95% dimethyl poly-siloxane) capillary column. The injector and detector temperatures were 210°C and 280°C respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL/min; oven temperature programmed was 60-210°C at the rate of 3°C/min; finally held isothermally for 5 min.

GC-MS analysis was carried out using a Perkin Elmer Clarus 500 GC (Shelton, CT06484, USA), coupled with Perkin Elmer Clarus 500 mass spectroscopy fitted with RTA-5 (Restek, 5% Diphenyl/95% dimethyl poly-siloxane; 60 m × 0.32 mm, 0.25 µm film thickness) capillary column. The carrier gas was Helium (1 mL/min.). Injector temperature was 210°C and oven temperature was programmed 60-210°C at the rate of 3°C/min; finally held isothermally for 15 min. The ionization of the sample components was performed in EI+ mode of 70 eV.

The constituents of oil were identified by calculation of their retention indices under temperature programmed condition for *n*-alkanes (*C₈-C₃₀*). Identification of individual components was assigned by retention time comparison with authentic components and oil of known composition and by mass spectra with those obtained from Wiley/NIST/Pfleger Mass Spectral library (Version 2.0 f) as well as with literature data.

2.4 Repellent activity

During bioassay half filter paper discs (8 cm diameter) were treated with 5, 10 and 20 µL dosage of essential oil samples and dried. Half the bottom of a Petri dish (8 cm diameter) was covered with treated filter paper, while the other half was covered with an untreated half filter paper disc. Ten adults with mixed sex were taken from the reared culture and were introduced in the middle of each Petri dish, and the lid was closed. The assessment was carried out at 28 ± 2°C and 75 ± 5% RH, natural photoperiod. Experiments were carried out in triplicate so that 30 adults per concentration were assayed; the number of insects on the two half paper discs was recorded after 1 and 5 h from the beginning of the test. Additional trials in which the Petri dishes were not treated with essential oil were carried out in order to measure variability in insect distribution in the analysis method.

2.5 Fumigant toxicity

During fumigant toxicity test, pieces of Whatman filter paper (5 mm diam.) were impregnated by 5, 10 and 20 µL per 0.25 L air of each essential oil sample separately; each filter paper was then rapidly hung in the center of a 250 mL glass container containing 40 adults of *T. castaneum* with 100 g of wheat grains. A positive control was kept for comparison purposes. All the containers were made airtight and incubated for 24 and 48 h in three replicates at
28 ± 2°C and RH 75 ± 5%. After the stipulated exposure, the vapor was allowed to disperse for 30 min before the mortality rates in each treatment were determined. Test insects were identified as dead if their appendage did not move when prodded with a pin.

2.6 Fumigant toxicity of essential oil in the presence of wheat grains

The different samples (TNG, TNB and TNM) of essential oil of T. nubigenum were used to fumigate the wheat samples separately. Five hundred gram of fresh un-infested wheat sample (var. UP 262; 11.20% moisture) was kept separately in closed glass containers (1000 mL volume). Forty adults of T. castaneum with mixed sex were introduced in the containers. Required amount of the oil of each sample was introduced separately in the glass containers by impregnating in cotton swab (500 mg) to obtain concentration of 40 and 80 µl per 0.25 L. All the containers were sealed. The wheat sample inoculated with the test insects without oil treatment served as controls. Each set was kept in three replicates. After 6 months of storage at laboratory conditions in a temperature/humidity control cabinet (28 ± 2°C and RH 75 ± 5%) in darkness, the efficacy of different samples of T. nubigenum oil on insect infestation was determined by calculating grain damage (%), weight loss (%) and feeding deterrence (%) of treated and control sets. The grain damage was determined by counting feeding injuries on the surface of the grains. The weight loss (%) and Feeding Deterrence Index (FDI%) of the stored wheat samples were calculated by the following formula.

Weight loss of wheat grains (%) = W-W/w/W × 100, where W and w represents the weight of wheat grains before and after the experiment, respectively.41

Feeding deterrent index (FDI) [%] = C-T/C + T × 100, where C and T is the weight loss of wheat grains in the controls and in the fumigated sets, respectively.41

2.7 Effect of T. nubigenum essential oil samples on the germination rate of grains

Samples of wheat grains (500 g) were treated with higher dose (80 µl per 0.25 L air) of essential oil samples of T. nubigenum as described in previous experiments, but insects were not added to grains to see the effects of essential oil on germination of grains fumigated. And then, all glass containers were sealed and kept in incubators at 28 ± 2°C, 75 ± 5% RH for 6 months prior to determining germination rate. Stocks of 100 intact grains treated and untreated (6 months stored) as well as fresh healthy seeds were selected and placed onto blotter paper in Petri plates for germination separately. The blotter papers were soaked with distilled water at regular interval of 2 days. Germinated grains were counted after 7 days and results were given as a percentage. Experiments were carried out in triplicate.

2.8 Statistical analysis

All the mortality values were represented as % mortality ± standard error (SE). All mortality rates of treatment were corrected by the mortality rate of the control40. The lethal concentration (LC₅₀), lower and upper confidence limits (LCL-UCL), slope value, t ratio, g value, heterogeneity factor and chi-square values were calculated using computer software of Robertson et al.17. Correlation and linear regression analysis were carried out to define all dose relationships18. The statistical analysis was done by one-way analysis of variance (p > 0.05) and means are separated by Tukey’s multiple range tests (SPSS 10.0; Chicago, Ill., U.S.A.).

3 RESULTS

During the extraction of essential oils from different samples of T. nubigenum, TNB exhibited the highest yield percentage (0.6%) followed by TNM (0.5%) and TNG (0.3%) on dry weight basis. GC and GC-MS analysis of all the three samples indicated that, selin-11-en-4-ol (10.3%) and methyl acetoxyronone (9.5%) were as major components in TNG oil. Borneol (19.8%), p-menthene-1-ol (11.7%), 1,8-cineole (10.9%), cis-piperitol (10.9%) and bornyl acetate (8.1%) were found as major components in TNG oil while bornyl acetate (38.1%), borneol (19.5%), 1,8-cineole (7.3%) were identified as major constituents in TNM oil (Table 1).

The repellent effects of the oil samples are shown in Table 2 which showing the random insects distribution in control experiments (non-treated Petri plates), confirming the absence of a behavioral bias in the analysis system. All the three oil samples had significant repellent activity on T. castaneum as compared to control sets. Among them, TNG produced the strongest repellency, and mean numbers of adults recorded in the control half after 5 h exposure were 6.2, 7.2 and 8.3 adults at 5, 10 and 20 µl dosage respectively.

The fumigant toxicity of all the essential oil samples of T. nubigenum adults is shown in Table 3. The fumigant toxicity tests indicated that all the essential oil samples had a remarkable fumigant activity on T. castaneum adults. Mortality of insect increased with increase dose and exposure time of the oil. Among three oil samples tested, TNG resulted in the highest mortality at all the concentrations and the mortality of T. castaneum exposed to TNG for 48 h were 100, 62.50 and 32.10% at dose of 20, 10 and 5 µl per 0.25 L air respectively. TNM was more toxic with 94.66% mortality than TNG at 20 µl/0.25 L air dose and 48 h of exposure. Table 4 represents that lethal concentration required to kill 50% of the test insect (LC₅₀) for TNG, TNB and TNM at 24 and 48h of exposure was 13.23 & 8.32, 15.72 & 9.22 and 14.22 & 8.82 µL per 0.25 L air respectively.
ly. The \( t \) ratio values were greater than 1.96, showing a significant regression of each dose response line. The heterogeneity factor was \( \leq 1.0 \), indicating that the log-dose-probit lines are within 95% confidence limits. The \( g \) value was found to lower than 0.5 showed that mean was within the limit at all probability levels of 90, 95 and 100 \( \% \) (Table 4). On regression analysis of the observed data, concentration dependent significant correlation of the essential oil with adult mortality of \( T. castaneum \) \( (p \leq 0.01) \) was found.

Bioassays in present of wheat grains showed that all the samples of \( T. nubigenum \) essential oils significantly protected the stored grains from biodeterioration by \( T. castaneum \). (Table 5). TNG essential oil had the highest feeding deterrenccy index against \( T. castaneum \) (91.74 & 94.15\%) followed by TNM (83.02 & 89.33\%) and TNB (75.92 & 83.02\%) essential oils at 40 and 80 \( \mu \)L per 0.25 L air dosage respectively. There were 83.21\% grains damaged and 45.82\% weight loss in control set. However, significant reduction in weight loss was found in fumigated wheat grains against the test insect.

The fumigated grains had more than 85\% of seed germination rate and the differences were not significant by comparing with germination rate of fresh healthy seeds \( \text{(Control-2)} \). However, significant reduction in germination rate was found in fumigated set-1 \( \text{(six months stored grains) where only 38.82\% seed germination was observed (Fig. 1).} \n
| Compounds                | Retention index | TNG  | TNB  | TNM  |
|--------------------------|-----------------|------|------|------|
| \( \alpha \)-Pinene       | 939             | 1.0  | 1.1  | 1.4  |
| Camphene                 | 954             | 1.6  | 1.5  | 2.3  |
| Sabinene                 | 975             | 4.9  | 2.3  | 1.0  |
| \( p \)-Cymene           | 1025            | 2.4  | 1.5  | 1.5  |
| 1, 8-Cineole             | 1033            | 2.1  | 10.9 | 7.3  |
| Linalool                 | 1097            | 0.7  | \( \text{=} \) | \( \text{=} \) |
| Camphor                  | 1142            | 6.9  | 9.7  | 1.3  |
| Pinocarvone              | 1161            | \( \text{=} \) | 0.3  | 0.4  |
| Borneol                  | 1169            | 5.8  | 19.8 | 19.5 |
| Terpinene-4-ol           | 1177            | 7.1  | 2.2  | 1.2  |
| \( \alpha \)-Terpineol    | 1191            | 0.5  | 3.2  | 0.2  |
| Cis-piperitol            | 1194            | 0.1  | 10.9 | 0.1  |
| \( \gamma \)-Terpineol   | 1217            | 0.1  | 1.0  | 2.4  |
| Bornyl acetate           | 1289            | 0.7  | 8.1  | 38.1 |
| Thymol                   | 1290            | 1.1  | 1.1  | 0.1  |
| Aromadendrene            | 1435            | 2.7  | \( \text{=} \) | \( \text{=} \) |
| \( \beta \)-Farnesene     | 1457            | 3.7  | 0.6  | 2.2  |
| Germacrene-D             | 1485            | 2.5  | 0.2  | 0.5  |
| 2,6,8-Trimethyl-4-nonanone| 1498           | 8.8  | \( \text{=} \) | \( \text{=} \) |
| Nerolidol                | 1563            | 2.2  | 0.1  | 0.3  |
| Caryophyllene oxide      | 1583            | 1.1  | 0.3  | 0.9  |
| Globulol                 | 1585            | 3.0  | 0.2  | 0.2  |
| \( \beta \)-Cudesmol     | 1648            | 4.2  | 1.6  | 2.6  |
| Selin-11-en-4-\( \alpha \)-ol | 1658         | 10.3 | \( \text{=} \) | \( \text{=} \) |
| Methyl acetopyronone     | \( \text{=} \) | 9.5  | \( \text{=} \) | \( \text{=} \) |

TNG: \( T. nubigenum \) (Gothin); TNB: \( T. nubigenum \) (Burphu); TNM: \( T. nubigenum \) (Milam Glacier)
Table 2  Repellent effects of *T. nubigenum* essential oils on *T. castaneum* adults after different exposure times in the filter paper test.

| Essential oil Samples | Dose in µL per plate | Mean number of adults present on each half ± SE |
|-----------------------|-----------------------|-------------------------------------------------|
|                       |                       | 1h                                              | 5h                                      |
|                       |                       | T      | U       | Chi square | T      | U       | Chi square |
| TNG                   | 20                    | 1.3 ± 0.3a | 8.7 ± 1.0a | 5.8       | 1.7 ± 0.2a | 8.3 ± 0.4a | 4.7       |
|                       | 10                    | 2.5 ± 0.6a | 7.5 ± 0.5b | 2.9       | 2.6 ± 0.8b | 7.2 ± 0.3b | 2.4       |
|                       | 5                     | 3.2 ± 0.2a | 6.8 ± 0.4a | 1.2       | 3.8 ± 0.5c | 6.2 ± 0.6b | 1.1       |
|                       | 20                    | 2.7 ± 0.4b | 7.3 ± 0.8b | 3.2       | 2.9 ± 0.7b | 7.1 ± 1.2b | 2.5       |
| TNB                   | 10                    | 3.2 ± 0.5b | 6.8 ± 0.6c | 1.9       | 3.5 ± 0.5b | 6.5 ± 0.5b | 1.6       |
|                       | 5                     | 4.0 ± 0.2a | 6.0 ± 1.2c | 0.5       | 4.4 ± 0.6c | 5.6 ± 0.4c | 0.3       |
|                       | 20                    | 2.2 ± 0.4b | 7.8 ± 1.5b | 3.3       | 2.2 ± 0.2b | 7.8 ± 1.0b | 2.5       |
| TNM                   | 10                    | 2.6 ± 0.6c | 7.4 ± 0.5c | 1.8       | 2.8 ± 0.2b | 7.2 ± 0.5b | 1.6       |
|                       | 5                     | 3.8 ± 0.4c | 6.2 ± 0.6c | 0.7       | 4.4 ± 0.6c | 5.6 ± 0.9c | 0.9       |

Data are mean of three replicates ± Standard error; T: treated half, U: untreated half, Data followed by different letters (a, b, c, d) in the same column show significant differences, based on Tukey’s multiple range tests at 5% level of significance.

Table 3  Fumigant effects of different essential oil samples of *T. nubigenum* on *T. castaneum* adults.

| Essential oil Samples | Dose in µL per 0.25 L air | Mortality (%) ± SE |
|-----------------------|---------------------------|--------------------|
|                       |                           | 24 h               | 48 h               |
| TNG                   | 20                        | 96.50 ± 0.50a      | 100.00 ± 0.00a     |
|                       | 10                        | 46.78 ± 1.00b      | 62.50 ± 0.31b      |
|                       | 5                         | 24.60 ± 0.32c      | 32.10 ± 1.20a      |
|                       | 20                        | 84.50 ± 0.90a      | 92.72 ± 1.14a      |
| TNB                   | 10                        | 40.48 ± 0.35b      | 54.56 ± 2.00b      |
|                       | 5                         | 20.50 ± 1.15c      | 26.23 ± 1.13c      |
|                       | 20                        | 90.23 ± 2.00a      | 94.66 ± 2.00a      |
| TNM                   | 10                        | 42.50 ± 1.60b      | 58.32 ± 0.91b      |
|                       | 5                         | 22.24 ± 0.40c      | 28.50 ± 0.27c      |

Data followed by different letters (a, b, c) in the same column show significant differences, based on Tukey’s multiple range tests at 5% level of significance.

Table 4  Summary of the toxicity of essential oil samples of *T. nubigenum* against adults of *T. castaneum*.

| Essential oil Samples | Exposure period (h) | LC50 (µL per 0.25 L air) | LCL-UCL | g Valuea | t ratioa | Heterogeneitya | Chi square |
|-----------------------|--------------------|--------------------------|---------|----------|----------|---------------|------------|
| TNG                   | 24                 | 13.23                    | 9.75-15.24 | 0.201    | 3.53     | 0.19          | 2.36       |
|                       | 48                 | 8.32                     | 6.22-10.62 | 0.198    | 4.33     | 0.18          | 4.15       |
| TNB                   | 24                 | 15.72                    | 11.24-17.82 | 0.218    | 2.86     | 0.17          | 3.12       |
|                       | 48                 | 9.22                     | 7.39-11.02 | 0.205    | 3.23     | 0.17          | 2.85       |
| TNM                   | 24                 | 14.22                    | 10.23-16.55 | 0.209    | 3.21     | 0.19          | 2.15       |
|                       | 48                 | 8.82                     | 6.12-10.10 | 0.202    | 4.02     | 0.18          | 4.02       |

LCL and UCL are lower and upper confidence limit, g value, t ratio and heterogeneity were significant at all probability levels (90, 95, 99 %).
DISCUSSION

Fig. 1 Germination rate (mean ± SE) of wheat grains exposed in TNG (T. nubigenum Gothing); TNB (T. nubigenum Burphu) and TNM (T. nubigenum Milam Glacier) essential oils (80 µL per 0.25 L air) after 6 months of fumigation. Mean followed by same letter are not significantly different by Tukey’s multiple range tests at 5% level of significance.

Table 5 Efficacy of T. nubigenum essential oils in protection of wheat grains from infestation.

| Essential oil Samples | Dose (µL per 0.25 L air) | Weight loss (%) | Grain damaged (%) | FDI index (%) |
|-----------------------|--------------------------|-----------------|-------------------|---------------|
| TNG                   | 40                       | 3.52 ± 0.50°    | 1.42 ± 0.50°      | 91.74         |
|                       | 80                       | 1.38 ± 0.12°    | 0.47 ± 0.10°      | 94.15         |
| TNB                   | 40                       | 6.27 ± 0.32°    | 2.12 ± 0.90°      | 75.92         |
|                       | 80                       | 4.25 ± 0.90°    | 1.28 ± 0.20°      | 83.02         |
| TNM                   | 40                       | 4.25 ± 0.75°    | 1.73 ± 0.50°      | 83.02         |
|                       | 80                       | 2.58 ± 0.15°    | 0.72 ± 0.02°      | 89.33         |
| Control               |                          | 45.82 ± 1.15°   | 83.21 ± 2.20°     |               |

Data followed by different letters (a, b, c) in the same column show significant differences, based on Tukey’s multiple range tests at 5% level of significance, FDI- Feeding Deterrency Index.

that among the three samples viz., TNG, TNB and TNM tested for insecticidal activity, TNG was more potent and order of potency during fumigant experiment were TNG > TNM > TNB. The overall findings evidenced that 20 µL per 0.25 L air dose of all the three tested samples is enough to cause <92% mortality of test insect within 48 h in space tests.

All the essential oil samples had significant repellent action against T. castaneum. The repellency of insects increased with the increase of oil concentration and exposure times and TNG was turn out as more potent candidate plant than TNM and TNB. Othira et al.\textsuperscript{21} reported that essential oil of Hyptis spicigera exhibited strong insect repellent activity at lower dosage against Sitophilus zeamais Motsch and T. castaneum and it was not significantly influenced by increase in the concentration of oil. On the contrary, Conti et al.\textsuperscript{20} reported that the essential oil of Hyptis spicigera exhibited a higher repellent effect in comparison to H. suaveolens against Sitophilus granarius and by increasing the dose of the oil the repellent activity was found to be increased. Similarly, in the present study all the dosage of TNG oil exhibited higher repellent activity than TNM and TNB essential oil samples and repellency increased with increasing oil concentration. These differences and similarities in repellent activity might be due to the essential oils used from different plants and variation in their chemical constituents. The repellent plants may contain certain active volatile compounds that elicit antifeedant behaviour by the visiting insects.

Present study indicated the insecticidal activity of all the tested samples were dose and time dependent. The mortality of insects increased with increasing oil concentration and exposure time which is parallel with the findings of earlier investigators\textsuperscript{18}. Our findings showed that lower concentrations with longer exposure achieved similar level of mortality than required when the oils were applied at higher dosage. Thus, in present bioassay exposure period
is considered to be most important factor affecting the toxic effects of the vapor of essential oils to a certain extent than the dosage. The present findings are supported by Conti et al. and indicate that insecticidal mode of action of the studied essential oil largely attributed to their fumigant action and found to be toxic by penetrating the insect body via respiratory system.

Plant products inter into the tissue and organ of target insects and affect the activity of various detoxifying enzymes. Francis et al. investigated the insecticidal mechanisms by determining the activity of detoxifying enzymes after biological insecticides have entered the target insects. There are two enzymes i.e. acetylcholinesterase and glutathione S-transferase related to the nervous system in insect body; and target pest die when the activity of these enzyme is inhibited at certain extent. These two enzymes generally have key functions in the interaction between insects and fumigants. Earlier workers found that natural fumigants caused a significant inhibition of these two enzymes. Hence, in the present study exposure of essential oil vapours might be causes inhibition of enzymes acetylcholinesterase and glutathione S-transferase present in the insects resulted the death of insects.

A perusal of research papers showed that the percentage of essential oil extracted and their chemical composition vary widely between the same species collected from different habitats. The present investigated plant species also showed remarkable variation in their yield and composition. Sample of T. nubigenum procured from Burphu (TNB, on the way of Milam Glacier) gave higher yield of essential oil followed by Milam Glacier (TNM) and Gothiting; Niti valley (TNG) samples. In addition, during GC and GC-MS analysis these 3 essential oil samples of T. nubigenum also showed variation in their chemical composition. Component selin-11-en-4-a-oi was found in higher amount in TNG essential oil, while TNB oil showed occurrence of borneol in high content. Meanwhile, bornyl acetate was reported in higher amount in TNM sample. In earlier investigation, chemical profile of T. nubigenum essential oil collected from Kumaun region (3600 m) of the Indian Himalaya exhibited the presence of sabine in higher amount. Similarly, Lohani et al. identified 38 compounds in T. nubigenum essential oil collected from alpine area of Uttarakhand with 1,8 cineole, sabine, eudesmol and camphor were as major constituents. However, the chemical profile of present studied samples did not show these compounds as major ones as described understory. These yield percent and composition variation in essential oils of same species from different habitats are due to differences in the geographical origin, time of extraction and plant part used for the extraction. Other factors such as genotype, chemo-type, environmental and agronomic conditions, can all influence the composition of the final natural product.

Plant essential oils are consisting of complex mixtures of monoterpenes and sesquiterpenes and the toxic properties of essential oils are associated with smaller group of the constituents, acting additively or synergistically. Earlier scholars investigated the monoterpenes and sesquiterpenes as major insecticidal agents in plant essential oils. Due to their high volatility nature they have significant fumigant action that might be of great consequence for stored product pests. The fumigant toxicity of 1, 8-cineole, camphor, eugenol, linalool, carvacrol, thymol, borneol, bornyl acetate has already been pointed out against stored product insects Sitophilus oryzae (L.), Rhyzopertha dominica (F.) and T. castaneum. So, the results of this study indicate that the toxic effect of different samples of T. nubigenum could be attributed to the presence of these components.

All the tested essential oil samples of T. nubigenum significantly protected the 500 g of wheat grains up to 6 months from insect infestation and order of their efficacy in grains protection was TNG > TNM > TNB. However, 500 ppm of Aegle marmelos oil significantly protected wheat grains up to 12 months from the infestation by R. dominica, S. oryzae and T. castaneum thus, more efficacious than the present studied oil samples. This might be due to the differences in plant species and long time constancy of their vapour in fumigation chamber. Experiment on fumigant effect of essential oil on seed germination indicated that there are no side effects on germination rate of grains exposed for 6 months of fumigation. Similar results were reported in our earlier research where no significant effects of Clausena pentaphylla and Chenopodium ambrosioides oils & their formulations on germination rate of pigeon pea seeds was found. These all observations imply that it is feasible for practical use as seed protectants.

To our knowledge, this is the first study on the insecticidal activity of T. nubigenum essential oil against T. castaneum. The above findings demonstrate that oil sample of T. nubigenum collected from Gothiting; Niti valley was more toxic than that of oil samples from Burphu and Milam Glacier of Uttarakhand Himalayas. In conclusion, essential oil from T. nubigenum can be implemented as botanical insecticide for the control of T. castaneum and can be useful for managing the red flour beetles in enclosed warehouses filled with grains. However, further work would be required to standardize its safety limit and organoleptic evaluation. This study will help the researchers to find more toxic species of T. nubigenum which provide a preliminary basis for the development of botanical insecticides of future application.

REFERENCES
1) Shaaya, E.; Kostjukovsky, M.; Menasherov, M.; Plotkin,
S. Essential oils and their components as active fumigants against several species of stored product insects and fungi. *Acta Hortic.* 344, 131-137 (1993).

2) Isman, M. B. Botanical insecticides, deterents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* 51, 45-66 (2006).

3) Pandey, A. K.; Palni, U. T.; Tripathi, N. N. Repellent activity of some essential oils against two stored product beetles *Callosobruchus chinensis* L. and *C. maculatus* F. (Coleoptera: Bruchidae) with reference to *Chenopodium ambrosioides* L. for the safety of pigeon pea seeds. *J. Food Sci. Technol.* 51, 4066-4071 (2014).

4) Liu, Z. L.; Ho, S. H. Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook f. et Thomas against the grain storage insects, *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst). *J. Stored Prod. Res.* 35, 317-328 (1999).

5) Kumar, R.; Kumar, A.; Prasad, C. S.; Dubey, N. K.; Samant, R. Insecticidal activity of *Aegle marmelos* (L.) Correa, essential oil against four stored grain insect pests. *Internet J. Food Saf.* 10, 39-49 (2008).

6) Yang, F. L.; Zhu, F.; Lei, C. L. Garlic essential oil and its major component as fumigants for controlling *Tribolium castaneum* (Herbst) in chambers filled with stored grains. *J. Pest Sci.* 83, 311-317 (2010).

7) Ambasta, S. P. The useful plants of India, Publishers National Institute of Science Communication Dr K S Krishnan Marg, New Delhi, (2006).

8) Joshi, R. K. Comparison of chemical composition of essential oil of *Tanacetum longifolium* from two different altitudes of Western Himalaya of Kumaun region of Uttarakhand, India. *Int. J. Herbal Med.* 1, 42-44 (2013).

9) Joshi, R. K.; Bisht, B. S. Antibacterial activity of volatile oil of *Tanacetum longifolium* from Western Himalayan region of Uttarakhand, India. *J. Nat. Prod. Plant Resour.* 2, 721-724 (2012).

10) Pandey, A. K.; Singh, P.; Mohan, M.; Tripathi, N. N. New reports on chemical composition of *Clausena pentaphylla* Linn. essential oil from India. *Chem. Nat. Comp.* 48, 896-897 (2012).

11) Adams, R. P. Identification of essential oil components by Gas Chromatography/Mass Spectrometry. 4th Ed. Allured Business Media, Carol Stream, IL, USA, (2007).

12) Cosimi, S.; Rossi, E.; Cioni, P. L.; Canale, A. Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pest: evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). *J. Stored Prod. Res.* 45, 125-132 (2009).

13) Huang, Y.; Lam, S. L.; Ho, S. H. Bioactivities of essential oil from *Elletaria cardamomum* (L.) Maton to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst.). *J. Stored Prod. Res.* 36, 107-117 (2000).

14) Parkin, E. A. Stored Product Entomology (the assessment and reduction of losses caused by insects to stored food stuffs). *Annu. Rev. Entomol.* 1, 233-240 (1956).

15) Isman, M. B.; Koul, O.; Lueynski, N. Insecticidal and antifeedant bioactivity of neem oil and their relationship to azadirachtin content. *J. Agric. Food Chem.* 38, 1406-1411 (1990).

16) Abbott, W. S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18, 265-267 (1925).

17) Robertson, J. L.; Russell, R. M.; Preisler, H. K.; Savin, N. E. Bioassay with arthropods POLO computer programme for the analysis of bioassay data, 2nd ed. CRC Press, Boca Raton, pp.1-224 (2007).

18) Sokal, R. R.; Rohlf, F. J. Introduction to biostatistics. Freeman WH, San Francisco, (1973).

19) Sahaf, B. Z.; Moharramipour, S. Fumigant toxicity of *Carum copticum* and *Vitex pseudo-negundo* essential oils against eggs, larvae and adults of *Callosobrachus maculatus*. *J. Pest Sci.* 81, 213-220 (2008).

20) Haouas, D.; Cioni, P. L.; Halima-Kamel, M. B.; Flamini, G.; Hamouda, M. H. B. Chemical composition and bioactivities of three *Chrysanthemum* essential oils against *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae). *J. Pest Sci.* 85, 367-379 (2012).

21) Othira, J. O.; Onek, L. A.; Deng, L. A.; Omolo, E. O. Insecticidal potency of *H. spicigera* preparations against *Sitophilus zeamais* (L.) and *Tribolium castaneum* (Herbst) on stored maize grains. *Afr. J. Agric. Res.* 4, 187-192 (2009).

22) Conti, B.; Canale, A.; Cioni, P. L.; Flamini, G.; Rifici, A. *Hypitisa suaveolens* and *Hypitida spicigera* (Lamiaceae) essential oils: qualitative analysis, contact toxicity and repellent activity against *Sitophilus granarius* (L.) (Coleoptera: Dryophthoridae). *J. Pest Sci.* 84, 219-228 (2011).

23) Li, S.; Li, M.; Huang, Y.; Hua, R.; Lin, H.; He, Y.; Wei, L.; Liu, Z. Fumigant activity of *Illicium verum* fruit extracts and their effects on the acetylcholinesterase and glutathione S-transferase activities in adult *Sitophilus zeamais*. *J. Pest Sci.* Doi 10: 1007/s10340-013-0520 (2013).

24) Francis, F.; Vanhælen, N.; Haubrege, E. Glutathione S-transferases in the adaptation to plant secondary metabolites in the *Myzus persicae* aphid. *Arch. Insect Biochem. Physiol.* 58, 166-174 (2005).

25) Ramsey, J. S.; Rider, D. S.; Walsh, T. K.; De Vos, M.; Gordon, K. H. J.; Ponnala, L.; Macmíl, S. L.; Roe, B. A.;
Fumigant efficacy of Tanacetum nubigenum essential oil

Jander, G. Comparative analysis of detoxification enzymes in Acyrthosiphon pisum and Myzus persicae. Insect Mol. Biol. 19, 155-164 (2010).

26) Ranson, H.; Prapanthadara, L.; Hemingway, J. Cloning and characterization of two glutathione S-transferases from a DDT-resistant strain of Anopheles gambiae. Biochem. J. 324, 97-98 (1997).

27) Barton, A.; Tjandra, J.; Nicholas, P. Chemical evaluation of volatile oils in Eucalyptus species. J. Agric. Food. Chem. 37, 1253-1257 (1989).

28) Beauchamp, P.; Dev, V.; Kashyap, T.; Melkani, A.; Mathela, C.; Bottini, A.T. Composition of the essential oil of Tanacetum nubigenum Wallich ex DC. J. Essent. Oil Res. 13, 319-323 (2001).

29) Lohani, H.; Chauhan, N.; Andola, H. Chemical composition of the essential oil of two Tanacetum species alpine region in Indian Himalaya. Nat. Acad. Sci. Lett. 35, 95-97 (2012).

30) Muller-Riebau, F.; Berger, B.; Yegen, O.; Cakir, C. Seasonal variations in the chemical compositions of essential oils of selected aromatic plants growing wild in Turkey. J. Agric. Food Chem. 45, 4821-4825 (1997).

31) Conde, R.; Corrêa, V. S. C.; Carmona, F.; Continic, S. H. T.; Pereira, A. M. S. Chemical composition and therapeutic effects of Lippia alba (Mill.) N. E. Brown leaves hydro-alcoholic extract in patients with migraine. Phytomedicine 18, 1197-1201 (2011).

32) Rice, P. J.; Coats, J. R. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern maize rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 87, 1172-1179 (1994).

33) Yang, F. L.; Li, X. G.; Zhu, F.; Lei, C. L. Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). J. Agric Food Chem. 57, 10156-10162 (2009).

34) Erler, F. Fumigant activity of six monoterpenoids from aromatic plants in Turkey against the two stored-product pests confused flour beetle, Tribolium confusum, and Mediterranean flour moth, Ephesia kuehniella. J. Plant Dis. Prot. 112, 602-611 (2005).

35) Rozman, V.; Kalinovic, I.; Korunic, Z. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. J. Stored Prod. Res. 43, 349-355 (2007).

36) Pandey, A. K.; Palni, U. T.; Tripathi, N. N. Evaluation of Clausena pentaphylla (Roxb.) DC oil as fungitoxicant against storage mycoflora of pigeon pea seeds. J. Sci. Food Agric. 93, 1680-1686 (2013).

37) Pandey, A. K.; Singh, P.; Palni, U. T.; Tripathi N. N. In vivo evaluation of two essential oil based botanical formulations (EOBBF) for the use against stored product pests, Aspergillus and Callosobruchus (Coleoptera: Bruchidae) species. J. Stored Prod. Res. 59, 285-291 (2014).