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**ORIGINAL ARTICLE**

# Toxic Effect of Cigarette Origin Tobacco Leaf (*Nicotiana tabaccum* L.) and Cigarette Smoke Extract on Germination and Bio-Chemical Changes of Bengal Gram (*Cicer arietinum* L.)

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A study has been conducted to observe the toxic effect of leachate from cigarette origin tobacco leaf and extracts of cigarette smoke on Bengal gram (*Cicer arietinum* L.). Results showed that germination index significantly vary with concentration. Almost all the treated seeds germinated within five days of incubation. However tobacco leaf extract showed 85 % germination with same days of incubation. The growth parameters like root length, shoot length, number of leaf per plant, shoot diameter, root and shoot fresh and dry weight etc. changes according the concentration gradient. The highest pigment content and germination index were recorded in treatment T4.

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*Key words: Cigarette smoke, pigment content, seed germination, tobacco leaf*

Toxicity of cigarette litter is well documented in previous literature (Moerman and Potts, 2011; Micevka *et al*., 2006). Basically this particular problem originates from the residual part of cigarette. Littered cigarettes are ubiquitous in the environment, and with global cigarette consumption currently on the rise, the global environmental burden of cigarette litter could become greatly exacerbated (Slaughter, 2010). The global environmental burden of cigarette litter is significant, as an estimated 4.5 trillion cigarettes are littered every year (Litter Free Planet, 2009). The study performed by Moriwaki *et al*.,(2009); Kitajima, & Katahira (2009) found that arsenic, nicotine, polycyclic aromatic hydrocarbons (PAHs), and heavy metals are released into the environment by littered "roadside waste" cigarette butts. Water channelled by sewer systems and streams acts to accumulate cigarette litter in localised areas and leach its chemical components into the environment (Moerman and Potts, 2011). However many researcher argued that a single piece of cigarette litter would not inflict serious environmental damage, the cumulative effect of many cigarette butts littered in a centralised area may present a significant threat to local organisms. But there are several studies focused on the toxicity of cigarette butts on aquatic animal (Baker *et al*.,

2004; Register, 2000; Slaughter *et al*., 2011). At the same time it was reported that water-soluble tobacco smoke extract from cigarettes inhibited seed germination of nine different species of higher plants (Bhalla and Sabharwal, 1973). On the other hand Micevka *et al*., (2006) suggest that the toxicity of cigarette butt leachates is in part due to heavy and trace metals. The occurrence of metals in cigarettes can largely be attributed to the growth and cultivation of tobacco (*Nicotiana tabacum*), as tobacco is known to readily accumulate metals from underlying soil (Tso, 1990). Chemically leachates are secondary metabolites having diverse group of alkaloids (Goetze *et al*., 2004) which seems to be associated with toxicity towards the growth of crops and weeds (Mafeo and Mashela, 2010; Jabran, 2010). Keeping in mind the above facts present work hypothesized that leaching of toxic chemicals from cigarette origin tobacco leave could interfere in germination process of Bengal gram (*Cicer arietinum* L.) and subsequently breakdown the pigment and delayed or barrier in synthesis of secondary metabolites

## MATERIALS AND METHODS

### Preparation of tobacco leaf extract and cigarette smoke extract

1.42 g tobacco leaves were taken from cigarette and mixed with 100 mL distilled water for overnight. Then the straw colour extract was filtered through Whatman 41 filter paper. This filtrate was considered as 100 % extract (Treatment $T_2$). Then the extract was diluted with distilled water for making 1:1 mixture (Treatment $T_3$). On the other hand one cigarette was burnt and entire smoke were dissolved in 100 mL distilled water (Fig. 1). After complete mixing of smoke in distilled water, the solution was considered as treatment $T_4$. Finally $T_4$ solution was make 1:1 dilution to make treatment $T_5$. A control ($T_1$) set up was considered using only distilled water. The entire treatment composition is presented in Table 1.

### Experimental treatment

Laboratory experiments with gram seedlings in Petri dishes were conducted in research laboratory of Department of Environmental Science, The University of Burdwan, Burdwan, West Bengal, India. Healthy seeds of Gram (*Cicer arietinum*) were collected from Kalna farm; Directorate of Agriculture, Govt. of West Bengal and the seeds were kept in airtight packets at room temperature and were used as experimental materials. After collection, seeds were surface sterilized in 0.1% $HgCl_2$ solution for 30 seconds, then the seeds were washed several times with tap water followed by distilled water. Fresh, clean, air dried Petri dishes (20 cm diameter) were taken and covered with filter paper discs. The filter paper discs were sprinkled with different treatment solution ($T_1$-control, $T_2$- 1.42 g tobacco leaf in 105 ml distilled water (100%), $T_3$-1:1 dilution of $T_2$ solution, $T_4$-mixing of the smoke, generated from complete burning of 1 cigarette, with 100 ml of distilled water, $T_5$-1:1 dilution of $T_4$ solution). 30 seeds were placed over the filter paper per discs sprinkled with respective treatment solution. The entire set up was then kept in a germination cage in a well ventilated and diffused sunlight mediated room. The ambient temperature of the experimental set up was kept at 22 $^0$C with one hour exposure to sunlight. The experiment was carried out according to the randomized block design with three replicates under laboratory condition. Each Petri dish containing the seeds was sprinkled with respective treatment solution at 2 days interval throughout the experimental period (10 days).

**Germination and growth attributes**

After 5th day of sowing of seeds the percentage germination value were considered and the length of shoot and root was recorded by centimeter scale using 10 day old seedlings.

Cumulative germination was determined by counting the number of germinated seed at 24 h intervals over a 120 h period and transformed into index of germination. The index of the following formula (Chiapuso, 1997).

GI = $(N_1)*1 + (N_2-N_1)*1/2 + (N_3-N_2)*1/3 + \ldots\ldots\ldots (Nn-Nn-1)*1/n$

Where N1, N2, N3, ……. Nn proportion of germinated seeds observed afterwards 1, 2, 3, n-1, n days. This index shows the delay in germination induced by the extract (15). At 10 days after sowing, shoot and root length of recipient species seedlings were measured.

**Seedling vigour index**

The seedling vigour index (SVI) was calculated by the following formula. SVI = germination percentage (shoot length + root length). The results of the experimental seedling were determined by counting the number of germinated seeds, measuring the whole length of seedlings in centimeter and weighing of seedling dry weight (after hot air oven dry at 80 $^0$C) and fresh weight in milligram.

**Chlorophyll assay**

Fresh young leaves (0.1g) were selected from plants under each treatment at the last day of the experiment, and washed with de-ionized water. The leaves were cut into small pieces. Chlorophyll fractions 'a', 'b' and total chlorophyll were determined in the acetone extract (80% v/v) (Arnon, 1949) measured in a spectrophotometer at 645, 652nm and 663 nm and the concentration were expressed as mg chlorophyll $g^{-1}$ fresh weight by using the following equations

$$Chl"a"(\mathrm{mgg^{-1}f.w}) = [12.7xD_{663} - 2.69xD_{645}]x\frac{vw}{1000}$$

$$Chl"b"(\mathrm{mgg^{-1}f.w}) = [22.9xD_{645} - 4.68xD_{663}]x\frac{vw}{1000}$$

$$TotalChl(\mathrm{mgg^{-1}f.w}) = D_{652}x1000x\frac{vw}{1000}$$

Where D = optical density; v = final volume of 80% acetone; w = weight of sample; f.w. =fresh weight of the sample

***Analysis of bio-chemical parameters***

**Estimation of Proline**

Proline was extracted from the leaves and estimated by the methods of Bates *et al*., (1973). Homogenates of the leaf samples were prepared in 3% sulphosalicylic acid. Pink color was developed by a reaction with glacial acid and ninhydrin. The color was separated in toluene layer and intensity of the color was measured at 529 nm., spectrophotometrically.

**Estimation of Soluble Sugars**

Soluble sugars were estimated by the method of Athanassova (Athanassova, 1996). Plant tissue (0.2 g) was homogenized in 2.0 mL of 80% ethanol (10% homogenate) using a Potter Elvehjem glass homogenizer and centrifuged at 3023g for 20 min. To 0.1 mL supernatant was added 0.9 mL water, 0.1 mL of 80% phenol, and 5.0 mL conc. $H_2SO_4$, and the mixture was allowed to stand at room temperature for 30 min. The absorbance was measured spectrophotometrically at 490 nm.

**Statistical Analysis**

Entire data were calculated with respect to control and other statistics at 95% confidential limits of upper confidence limit and lower confidence limit. A comprehensive statistical software package (SPSS 16.0) was used to calculate

ANOVA and DMRT test.

## RESULTS AND DISCUSSION

From the Table 2 it is clear that treatment T2 showed lower percentage of germination compared to the other treatments. On the other hand from the Fig. 2, it is observed that different treatment showed different number of seed germination in different day's interval. Almost all treatment showed 100 % germination within five days. But treatment T2 showed 85 % germination during first five days of sowing. The released alleochemicals probably interferes with the plant growth regulators (Mayer and Poljakoff-Mayer, 1963). The results indicate that the inhibition of germination is dependent on the concentration of extract from cigarette tobacco leaf and its entry through water soluble parts in to the growth (Suseelamma and Venkataraju, 1994). The most pronounce effects were recorded in T2 treatment. The germination index (GI) indicate that T2 is least compared to the control. But T4 treatment showed higher GI over control (Table 2). This is probably due to activation of some growth activating enzyme by the cigarette smoke mixed solution. Similar activation of seed germination was also reported by Bhalla *et al*., (1973).

When gram seedlings were treated with the concentrated (100 %) extract of both tobacco leaf and smoke extract, two different status of germination showed. In first case germination was delayed where as in second case germination accelerated. Similar acceleration of germination of tomato seeds by cigarettes smoke extracts was reported by (Bhalla, 1973). But at the same time germination status in cigarette smoke showed little slower rate compared to control. This is probably due to the chemical fraction which leads to the inhibition of germination (Bhalla, 1973).

The germination index value indicate all treatment significantly difference ($p<0.05$) from control (Table 2). The delay of germination due to abnormal secreation of indoleacetic acid (IAA) and Kinetic which are regulates the growth during seed germination (Mayer and Poljakoff-Mayer, 1963, Kochhar *et al*., 1970, Kochhar *et al*., 1971b, and Frey, 1999).

The variation of root length and shoot length was different in different treatment and all treatments were significantly different ($p<0.05$) from control (Table 2). Similar significant ($p<0.05$) difference in shoot length was also recorded in all treatments. But from both root and shoot length data it is clear that treatment T4 is distinctly different from other treatments. The reduction in vigour index of gram seedling may be due to reduced germination and shoot length, as vigour index is the product of germination and seedling length (Das *et al*., 2012). The variation of root length, shoot length, shoot branch/plant, root branch/p, number of leaf/p, shoot diameter and seed vigour index were all significantly decreased ($p<0.05$) compared to control with increasing the concentration of the extract from both leaf leachates of tobacco leaf and cigarette smoke extract (Table 2). From the fresh weight and dry weight plot (Fig. 3), it is demonstrated that fresh shoot weight always high compared to fresh root weight (Fig. 3). But the dry weight data signify that water retention was highest in T4 in comparison to other treatment but lower than control. The correlation table (Table 4) also revealed that dry weight of both root and shoot positively correlated ($p<0.01$) with seedling vigour index. Similar positive relationship of germination rate, root length, shoots length, biomass with seedling vigor index was also reported by Yazdani and Bagneri (Yazdani and

Bagheri, 2011). The biochemical response of the different treated plant also showed gradual reduction according to the concentration of the extract. The pigment content (Chl 'a', 'b' and total chl.) was highest recorded in treatment T5 followed by T3 and lowest in T4 (Table 3). The reduction of pigment is due to presence of Benzoic acid and phthalate which released from the smoke and tobacco leaf as an allelochemicals (Jianhua *et al*., 2012). Another possibility is that the allelochemicals may partially block the biosynthetic pathway of chlorophyll, or stimulate the degradative pathway of chlorophyll, or both, leading to a reduction of chlorophyll accumulation, in turn causing a reduction of photosynthesis and finally diminished total plant growth (Gibson and Liebman, 2003). On the other hand maximum reduction (92 %) of total sugar was recorded with tobacco smoke extract (T4). This is probably due to interference of photochemical in total sugar biosynthetic processes; this was also confirmed by Sing and Rao in rice (Singh and Rao, 2003). The free proline content in gram seedling is increased in all treatmens. About 66 % free proline increased in treatment T3 followed by 33 % and 25.5 % in treatment T4 and T5 respectively.

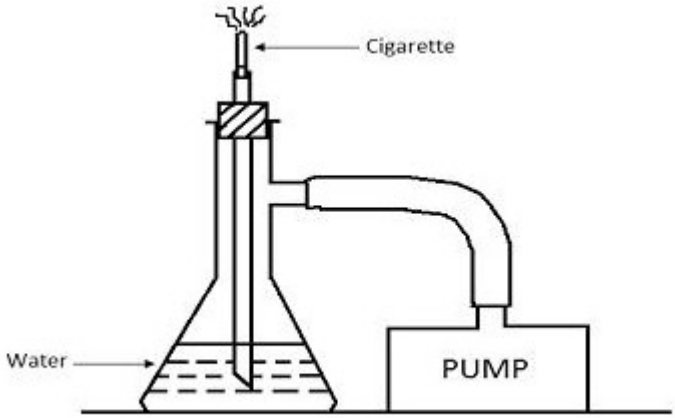


**Figure 1 :** Cigarette smoke extract

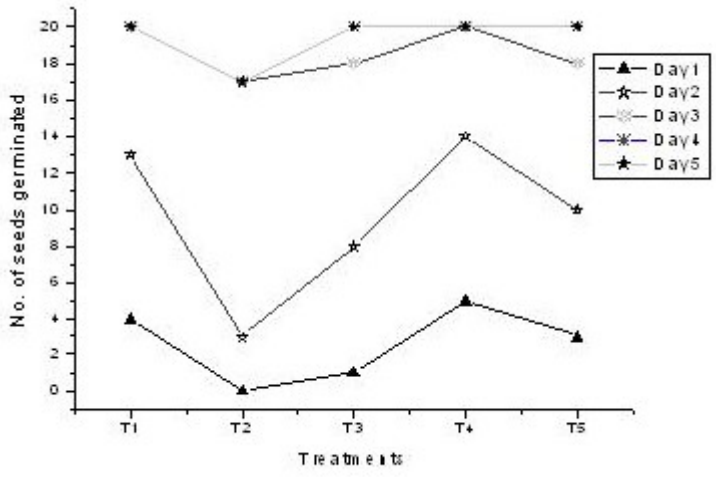


**Figure 2 :** Germination of seeds under different treatments

**Table 1 :** Treatment composition

| Treatment | Descriptions | % of germination after 3 days of sowing |
|---|---|---|
| $T_1$ | Control (only distilled water) | 100 |
| $T_2$ | 1.42 g tobacco leaf in 105 ml distilled water (100%) | 85 |
| $T_3$ | 1:1 dilution of $T_2$ treated solution | 100 |
| $T_4$ | Smoke from complete burning of one cigarette and the mixed the smoke with 100 ml distilled water | 100 |
| $T_5$ | 1:1 dilution of $T_4$ treated solution | 100 |

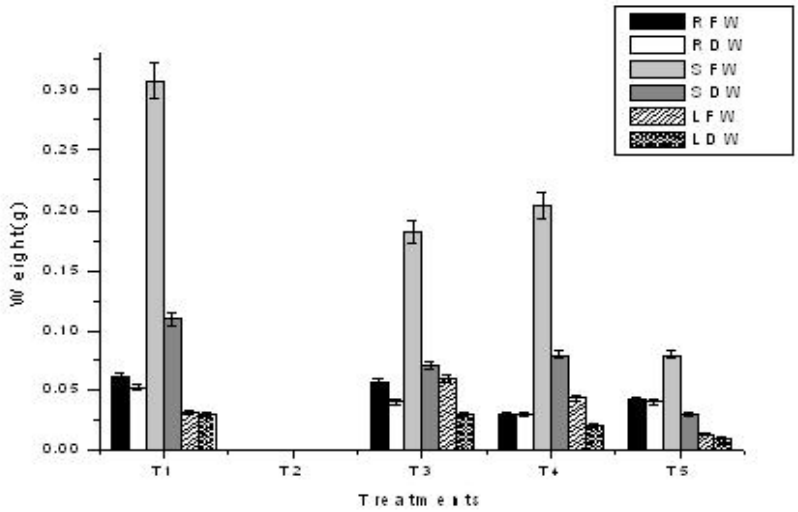


**Figure 3 :** Fresh weight and dry weight of root, shoot and leaves

**Table 2 :** Morphological parameters of gram seedlings after ten days of sowing under different treatments. Mean of three replicates ± SD

| Treat-ment | RL (cm) | SL(cm) | SB/ plant | RB/ plant | No. of leaf/ plant | S dia. (cm) | SVI | GI |
|---|---|---|---|---|---|---|---|---|
| $T_1$ | 9.43 ± 0.11$^b$ | 20.13 ± 0.63 $^a$ | 2 ± 0.22$^a$ | 3±0.02$^b$ | 17±0.246$^b$ | 0.85 ± 0.10$^a$ | 2956.6 ± 3.46$^a$ | 8.5 ± 0.476$^a$ |
| $T_2$ | -- | -- | -- | -- | -- | -- | -- | 6.17 ± 0.228$^c$ |
| $T_3$ | 5.8 ± 0.223$^c$ | 10.97 ± 1.04 $^c$ | 1±0.24$^b$ | 4±0.003$^b$ | 25±0.144$^a$ | 0.6 ± 0.36$^b$ | 1676.6 ± 2.558$^c$ | 7.83 ± 0.642$^b$ |
| $T_4$ | 12.2 ± 1.36$^a$ | 15.87 ± 0.006$^b$ | 3 ± 0.11$^a$ | 9 ±0.225$^a$ | 23±2.07$^a$ | 0.75 ± 0.116$^a$ | 1806.6 ± 0.003$^b$ | 9.5 ± 0.007$^a$ |
| $T_5$ | 3.16 ± 0.36$^d$ | 6.67 ± 0.033$^d$ | 3 ± 2.1$^a$ | 3±1.33$^b$ | 18±2.34$^b$ | 0.9 ± 0.81$^a$ | 983.2 ± 0.247$^d$ | 7.0 ± 0.32$^b$ |

Note: RL (root length), SL (shoot length), SB (shoot branch), S dia. (shoot diameter), SVI (seedling vigour index) and GI (germination index). Different letters indicate significant differences at p<0.01 according to the Tukey-HSD.

**Table 3 :** Biochemical changes of gram seedlings after …. days of sowing under different treatments. Mean of three replicates ± SD

| Treatment | Chl.'a' | Chl.'b' | Total chl. | Sugar (mg/g) | Proline (mg/g) |
|---|---|---|---|---|---|
| $T_1$ | 0.339±0.46$^a$ | 0.433±0.365$^a$ | 1.212±0.11$^a$ | 12.30±0.147$^a$ | 0.098±0.002$^c$ |
| $T_2$ | -- | -- | -- | -- | -- |
| $T_3$ | 0.216±0.79$^c$ | 0.221±0.021$^c$ | 0.683±0.024$^c$ | 1.251±0.269$^c$ | 0.163±0.050$^a$ |
| $T_4$ | 0.211±0.007$^c$ | 0.222±0.001$^c$ | 0.569±0.16$^c$ | 1.204±0.366$^c$ | 0.131±0.009$^b$ |
| $T_5$ | 0.312±0.096$^b$ | 0.345±0.096$^b$ | 0.892±0.013$^b$ | 6.231±0.723$^b$ | 0.123±0.043$^b$ |

Different letters indicate significant differences at p<0.01 according to the Tukey-HSD.

Note: Chl.'a': Chlorophyll 'a', Chlorophyll 'b', Total chl.: Total Chlorophyll

**Table 4 :** Correlation between growth physiology, Total Chlorophyll and Proline

| | RL | SL | RFW | SFW | SVI | TChl | Proline | RB/P | SD | RDW | SDW | LFW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SL | 0**.830** | | | | | | | | | | | |
| RFW | **0.801** | 0.335 | | | | | | | | | | |
| SFW | 0.703 | **0.968** | 0.138 | | | | | | | | | |
| SVI | 0.613 | **0.947** | 0.019 | **0.987** | | | | | | | | |
| TChl | -0.152 | 0.377 | -0.618 | 0.447 | 0.585 | | | | | | | |
| Proline | -0.257 | -0.523 | 0.048 | -0.428 | -0.533 | -0.780 | | | | | | |
| RB/P | 0.743 | 0.244 | **0.987** | 0.066 | -0.067 | -0.730 | 0.207 | | | | | |
| SD | -0.137 | 0.012 | -0.152 | -0.106 | 0.019 | 0.613 | -0.853 | -0.285 | | | | |
| RDW | 0.166 | 0.673 | -0.456 | **0.818** | **0.874** | 0.750 | -0.397 | -0.511 | -0.024 | | | |
| SDW | 0.752 | **0.971** | 0.213 | **0.996** | **0.967** | 0.361 | -0.368 | 0.149 | -0.172 | **0.772** | | |
| LFW | 0.273 | 0.061 | 0.308 | 0.143 | 0.004 | -0.678 | **0.819** | 0.432 | -**0.987** | -0.034 | 0.218 | |
| LDW | 0.387 | 0.656 | -0.112 | **0.807** | 0.764 | 0.202 | 0.120 | -0.091 | -0.592 | **0.799** | **0.817** | 0.566 |

**Bold value** indicate significance level $p < 0.01$; RL: root length; SL: shoot length; RFW: root fresh weight; SFW: shoot fresh weight; SVI: shoot vigor index; TChl: total Chlorophyll; RB/P: root branch per plant; SD: shoot diameter; RDW: root dry weight; SDW: shoot dry weight; LFW: leaf fresh weight

## CONCLUSION

Finally it can be concluded that cigarette origin tobacco leaf extract accelerate the growth parameter specially root hair and shoot length, but reduced the pigment content in leaf. However, highest pigment content and germination index were recorded in treatment T4 where smoke of burning cigarette dissolved in water.

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