Methyl bromide alternatives for presowing fumigation in tobacco seedling production

Özhan BOZ*, Ayhan YILDIZ, Kemal BENLİOĞLU, Hatice Seher BENLİOĞLU
Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, 09100 Aydın - TURKEY

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Abstract: This study aimed to evaluate the effects of soil fumigant alternatives to methyl bromide (MeBr) on the weeds, damping-off, and seedling growth of tobacco. Methyl bromide (90 g m⁻²), dazomet (D) (50 g m⁻²), and metam sodium (MS) (100 mL m⁻²) were evaluated in 2 locations in the Karacasu district of Aydın province, Turkey, February-April 2005. In addition, half doses of D and MS were investigated in 2006. MeBr treatment had 52.5% control against the postemergence damping-off caused by *Pythium* spp., while alternative treatments had no effect on the disease in 2005. In 2006, MeBr and MS (100 mL m⁻²) showed 77.6% and 100% control against the disease, respectively. All fumigant treatments significantly controlled pigweed species (*Amaranthus* spp.), common purslane (*Portulaca oleracea* L.), common lambsquarters (*Chenopodium album* L.), nettleleaf goosefoot (*Chenopodium murale* L.), burning nettle (*Urtica urens* L.), and large crabgrass (*Digitaria sanguinalis* (L.) Scop.), except dazomet (50 g m⁻²) on pigweed (86.2%), common lambsquarters (68.6%), nettleleaf goosefoot (60.5%), and burning nettle (64.3%) at one location in 2005. Similarly, all fumigants, including low doses of MS (50 mL m⁻²) and D (25 g m⁻²), significantly decreased the populations of pigweed species, common purslane, common lambsquarters, prostrate knotweed (*Polygonum aviculare* L.) and sowthistle species (*Sonchus* spp.) in 2006. Tobacco (cv. Akhisar) seedling height and fresh weight increased significantly with treatments of MeBr, MS (100 mL m⁻²), and D (50 g m⁻²) at the 2 locations in both years.

Key words: Damping-off, dazomet, metam sodium, methyl bromide alternatives, tobacco, weed

Tütün fidesi üretiminde ekim öncesi metil bromür alternatifleri

Özet: Bu çalışmadı, metil bromür (MeBr) alternatif toprak fumigantlarının ürün fide gelişimine etkisinin araştırılması amacıyla ele alınmıştır. 2005 ve 2006 yılları Şubat-nisan ayları boyunca Aydın ili Karacasu ilçesinde iki lokasyonda, ilk yıl MeBr (90 g m⁻²), dazomet (D) (50 g m⁻²) ve metam sodıyum (MS) (100 mL m⁻²), ikinci yılda ise bunlara ek olarak D ve MS’un yarısı dozlarını etkisi araştırılmıştır. 2005 yılında *Pythium* spp.”nın neden olduğu çökerten üzerine altrantatif uygulamaların hiç bir etkisi yokken, MeBr uygulaması % 52.5 oranında etkili bulunmuştur. 2006 yılı denemelerinde ise MeBr % 77.6, metam sodıyum’un 100 mL m⁻² dozu % 100 oranında çökertene etki göstermiştir. 2005 yılında bir lokasyonda dazomet’in (50 g m⁻²) horoz ibiğine (% 86.2), sirkene (% 68.6), duvar kazayağına % 60.5, isrgana olan % 64.3”lük etkinisinin dışında, tüm fumigantlar horoz ibiği türleri, semiz otu, sırken, duvar kazayağı, isrgan otu ve çatal otuna % 90dan fazla oranda etkili bulunmuştur. Benzer şekilde 2006 yılında, MS’un m⁻² ye 50 mL’lik ve D’in m⁻² ye 25 g’lik dozları da dahil olmak üzere tüm fumigantlar iki lokasyonda da horoz ibiği türleri, semiz otu, sırken, çoban değeği ve esek marulu türlerini önemli ölçüde azaltmıştır. Her iki yılda da MeBr, MS’un m⁻² ye 100 mL ve D’in m⁻² ye 50 g doz uygulamaları tütün fidesi boyunu ve yaş ağrılığını önemli derecede artırmıştır.

Anahtar sözüklər: Çökerten, dazomet, metam sodıyum, methyl bromür alternatifleri, tütün, yabancı ot

* E-mail: ozhanboz@yahoo.com
Introduction

After signing the Montreal Protocol to globally phase out the use of substances that contribute to the depletion of the ozone layer in 1987, Turkey decided to ban the use of methyl bromide (MeBr) by 2007. Over the past decade, researchers in Turkey have carried out 4 projects investigating the use of MeBr alternatives in agriculture. The first was entitled “Alternatives to the Use of Methyl Bromide as Soil Fumigants in Protected Horticulture and Ornamental Crops in the Western Mediterranean Region” (supported by UNIDO), and the research was conducted between 1998 and 2000. The second, “Introduction of Methyl Bromide Alternatives for Strawberry, Pepper and Eggplant Production in Turkey,” was conducted on strawberries in Aydın province and on peppers and eggplants in the eastern Mediterranean region between 1999 and 2002, with the support of the World Bank (Benlioğlu et al. 2001, 2002). The third was a project to phase out methyl bromide in the dried fig sector, supported by the World Bank between 2001 and 2004 (Meyvaci et al. 2003). The final project was entitled “Phase-Out of Methyl Bromide for Soil Fumigation in Protected Horticulture and Cut-Flower Production in Turkey” and was implemented by UNIDO between 2001 and 2004.

However, no research has been conducted on MeBr alternatives in tobacco production in Turkey (Anonymous 2008). For tobacco seedling production, there are approved and feasible non-MeBr alternatives in other countries, such as metam sodium (MS), dazomet (D), and Bunema in Greece (Chryssochoou 1999); MS, chloropicrin, 1,3-dichloropropene (1,3-D), 1,3-dichloropropene plus 17% chloropicrin (1,3-D+C-17), and 1,3-dichloropropene plus 35% chloropicrin (1,3-D+C-35) in the USA (Csinos et al. 1997, 2000); the floating system with expanded polystyrene (EPS) trays seeded with tobacco in Australia (Mattner et al. 2007), Malawi (Chilembwe et al. 2005), Argentina (Salles et al. 2001), and Brazil (Salles 2001); and 1,3-dichloropropene (1,3-D) plus chloropicrin, D, and MS in Spain (Bello et al. 2001).

Tobacco is the major crop in Karacasu (600 m altitude), Aydın province, Turkey, and its cultivation requires the production of healthy and vigorous transplants. Seedling production occurs in the 3 months from February to April, when temperatures are lower than the optimum for tobacco growth, over an area of 15 ha in 22 villages. Transplants need to be grown in raised seedbeds under unheated plastic tunnels that are approximately 1.5 m in width by 20-30 m in length by 1 m in height. Tobacco growers who produce their tobacco transplants in seedbeds should do so only in fumigated soil to reduce the risks of carrying soilborne diseases into the field and to control weeds. MeBr consumption is estimated to be around 9.1 metric tonnes for tobacco in the province.

This work was initiated to evaluate potential fumigant alternatives (MS and D) to MeBr fumigation showing promising results in some other countries, such as Spain (Bello et al. 2001) and Greece (Chryssochoou 1999), for tobacco seedling production in Aydın province, Turkey.

Materials and methods

The research was conducted in 2 villages (Ataköy and Ataeymir) in which tobacco is grown as a primary income source, in Karacasu, Aydın province, in 2005 and 2006. The experimental conditions, such as application time of fumigants, aeration, and transplanting time, were arranged according to the traditional tobacco seedling growing procedure of Karacasu district. A randomised block design with 3 or 4 replications for each experiment was used. Initially, raised bed plots were deep-cultivated (to a depth of 0.25 m), and the soil was then formed into beds measuring 1.5 x 30 x 0.30 m with furrows (0.5 m wide) separating the beds. Small raised beds (1.5 x 1.5 m) of dry farmyard manure, sieved by a commercial 5 mm screen, were also built for topdressing each of the treated plots. All treatments were applied on 26 January 2005 at the following rates: D (Basamid-BASF, 99% granular soil fumigant) at 50 g m⁻², MS (Doğal Kimya, Sniper fluid, 50% fluid) at 100 mL m⁻², and MeBr (Metabrom-Hektaş, 98% gaseous soil sterilant) at 90 g m⁻². Based on the previous year’s results, the same treatments and half amounts of D and MS were also administered on 3 February 2006. Dazomet was scattered homogenously on the surface of each plot with an ordinary flour sieve and irrigated with 5 L m⁻² of water after the materials were incorporated into the soil to a depth of 5-6 cm with a
garden rake. Metam sodium was applied to the soil surface via a hand sprayer in 1.5 L of water per square meter. Treated plots were immediately covered with polyethylene film (0.11 mm thick). Methyl bromide canes were placed into the soil of the plot, and they were burst after covering the beds with polyethylene sheets. The small raised beds for the topdressing were treated with the same fumigant as the main plots. Untreated control plots received water at comparable rates and were covered with polyethylene film. The polyethylene film was removed from all plots after 12 days in 2005 and after 7 days in 2006 to allow aeration.

Tobacco (cv. Akhisar) seeds whose germination rate had already been tested on water agar plates were sown on 8 March 2005 and on 2 March 2006, depending on the phytotoxicity tests. Immediately after sowing, each planting bed was covered with a 2-3 cm layer of topdressing material, which was subjected to the same treatment as the planting bed and irrigated with hand sprinklers. After seeding, the planting beds were covered with low plastic tunnels (1.5 m wide and 50 cm high).

In both years, soil temperatures at a depth of 10 cm were recorded hourly in the treated plots, from the onset of fumigation to the transplanting date of seedlings, using HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA).

Phytotoxicity tests

The phytotoxicity and persistence of fumigants in the soil was predicted by cress (Lepidium sativum L.) germination after polyethylene film removal. Soil samples (500 g) from a 0-15 cm profile depth from 4 or 5 different sites at each experimental plot were placed in 500 mL plastic pots, and the cress seeds (100 per pot) were immediately sown by hand. The pots were kept outdoors under natural climate conditions in a net house, irrigated when necessary, and examined daily. The test was finished within 10 days (±2 days) after about 50% of the control seedlings had emerged, and the germination rate was measured. This procedure was repeated 3 or 4 times every 5-7 days in 2005 and 2006, respectively. Tobacco seeds were sown when the percentage of germinated cress seeds in soil samples from treated plots remained at roughly the same rate as that in samples from untreated plots.

Treatment effects on damping-off, weeds, and seedling vigour

Damping-off

Three weeks after sowing, the percentage of postemergence damping-off was evaluated by calculating the percentage of total infected area showing damping-off symptoms in each experimental plot. Since damping-off is often seen in round or elliptical patches in seedling flats, the infected area was calculated using the elliptical area formula (S = \( \pi ab \); a: semimajor axis, b: semiminor axis).

Three to five randomly selected plants from affected areas in each plot were carefully dislodged and visually examined for damping-off symptoms. Based on initial observations, small pieces of necrotic root or crown tissue were excised aseptically and placed on potato dextrose agar (PDA) and water agar (WA). After incubation in an incubator at 21 °C for 3-7 days, the cultures were characterised and identified microscopically.

Weeds

The effect of treatments on the emergence of native weed populations was evaluated by counting all weed species on each plot 3 weeks after tobacco sowing. The species and number of weeds were counted in 4 random quadrates of 0.25 m² from each plot, and weeds were removed by hand after counting. The average number of weed species for each treatment was given as weed emergence per m².

Seedling vigour

Randomly selected seedlings (4 × 25 per plot) were dug up on 21 April 2005 and 2006. Transplant lengths and fresh weights were measured and averaged for subsequent comparisons of plant vigour.

Data analysis

Data were first analysed by analysis of variance, followed by mean separation using Fisher’s protected least significant difference test. Data calculated as percentages were arcsine-transformed prior to analysis. All analysis was performed with the JMP IN program (SAS Institute, 2004) at P ≤ 0.05.
Results

Daily mean soil temperatures at a depth of 10 cm under the polyethylene film during fumigation were in the range of 5.3-15.4 °C (average: 10.9 °C) in 2005 and 4.6-6.2 °C (average: 5.4 °C) in 2006. Soil temperatures at a depth of 10 cm during the period from the removal of the polyethylene films to the sowing of tobacco seeds averaged 7.6 °C in 2005 and 7 °C in 2006 (Figure 1). Rather cool temperatures dominated during the middle 2 weeks of February 2006, while the temperatures during the same period were higher in 2005.

Phytotoxicity tests

Phytotoxicity tests showed that there were significant differences in the germination percentages of cress seeds, except final counts (Table 1), following treatments in 2005 and 2006. Generally, the germination rate in MeBr-treated soil was found to be almost the same as that of the untreated control, while D and MS treatments caused significantly lower germination rates than those observed in the untreated control in 2005 and 2006. However, there were no significant differences in the germination rate for the treatments 3 and 4 weeks after film removal at the final counts in 2005 and 2006, except for D at 50 g m⁻² (P = 0.67 and 0.13).

Table 1. Average germination percentage of cress seeds in soil samples taken from experimental plots in Ataköy, 2005, and Ataeymir, 2006.

<table>
<thead>
<tr>
<th>Treatments *</th>
<th>2005**</th>
<th>2006**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 Feb.</td>
<td>15 Feb.</td>
</tr>
<tr>
<td>Untreated control</td>
<td>24.7 a</td>
<td>73.3 a</td>
</tr>
<tr>
<td>MeBr (90 g m⁻²)</td>
<td>7.7 b</td>
<td>70.3 a</td>
</tr>
<tr>
<td>MS (100 mL m⁻²)</td>
<td>2.0 b</td>
<td>41.3 b</td>
</tr>
<tr>
<td>MS (50 mL m⁻²)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D (50 g m⁻²)</td>
<td>1.3 b</td>
<td>27.7 b</td>
</tr>
<tr>
<td>D (25 g m⁻²)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*MeBr: Methyl bromide; MS: Metam sodium; D: Dazomet
**Means in columns followed by the same letter are not significantly different (P ≤ 0.05).
Damping-off

The germination rate of tobacco seeds was approximately 85% in both years. Typical postemergence damping-off symptoms occurring in patches and causing wet rot of roots and stems were observed in the experimental plots. When plated on WA or PDA, tissues from the diseased roots of sampled seedlings yielded primarily *Pythium* spp., and, to a lesser extent, *Rhizoctonia solani* (J.G. Kühn). MeBr treatment had 52.5% control against postemergence damping-off caused by *Pythium* spp., while alternative treatments had no effect on the disease in 2005. In 2006, MeBr and MS (100 mL m\(^{-2}\)) showed 77.6% and 100% control against the disease, respectively (Table 2).

Weeds

Eight different weed species were evaluated in the experimental plots. Generally, all fumigant treatments controlled weeds by more than 90%, including pigweed species (*Amaranthus* spp.), common purslane (*Portulaca oleracea* L.), common lambsquarters (*Chenopodium album* L.), nettleleaf goosefoot (*Chenopodium murale* L.), burning nettle (*Urtica urens* L.), and large crabgrass (*Digitaria sanguinalis* (L.) Scop.), excluding D treatment (50 g m\(^{-2}\)) on pigweed (86.2%), common lambsquarters (68.6%), nettleleaf goosefoot (60.5%), and burning nettle (64.3%) at the Ataeymir location in 2005 (Tables 3 and 4). In 2006, all treatments that included low dosages of MS (50 mL m\(^{-2}\)) and D (25 g m\(^{-2}\))...
significantly decreased the population of weed species such as pigweed species, common purslane, common lambsquarters, prostrate knotweed (*Polygonum aviculare* L.), and sowthistle species (*Sonchus* spp.) when compared to the untreated control (*P* = 0.0001) (Tables 5 and 6).

**Seedling vigour**

The plant height of cv. Akhisar significantly increased following the treatments with MeBr (90 g m$^{-2}$), D (50 g m$^{-2}$), and MS (100 mL m$^{-2}$) in 2005 and 2006, as well as the low-dose treatment of MS (50 mL m$^{-2}$) in 2006 (*P* = 0.001 and 0.005) (Table 7). Tobacco seedling fresh weights increased significantly after the treatments with MeBr, MS (100 mL m$^{-2}$), and D (50 g m$^{-2}$) in both years (*P* = 0.03 and 0.05).

**Discussion**

Metam sodium and D are recognised precursors to the formation of methyl isothiocyanate (MITC) in soils (Tomlin 2003; Ruzo 2006). The degradation of MITC is influenced by soil temperature, organic C content, moisture content, and texture (Smelt and Leistra 1974; Gerstl et al. 1977; Smelt et al. 1989; Boesten et al. 1991; Dungan et al. 2002). Of these soil conditions, temperature and organic C content often have the largest influence on MITC degradation (Dungan and Yates 2003). Our data from soil temperatures and cress germination tests (Figure 1 and Table 1) revealed that there was a time-varying and temperature-dependent risk for phytotoxicity 3 and 4 weeks after MS and D applications in 2005 and 2006.
For MeBr, the risk for phytotoxicity was quite low, even after 1 week of treatment under the low soil temperature conditions in planting beds in 2006. Fumigation for 12 days with D (50 g m⁻²) and MS (100 mL m⁻²) under winter conditions (mean soil temperatures: 10.9 °C) and tobacco seeding within 30 days after aeration provided no effective control against damping-off in 2005 (Figure 1 and Table 2). However, 7-day application of MS (100 mL m⁻²) when the mean soil temperature was 5.4 °C and the seeding was performed more than 20 days after aeration resulted in the same effect on damping-off as the application of MeBr (90 g m⁻²) in 2006. Csinos et al. (1997) indicated that fumigation of 32 days with D (38.7 g m⁻²) and fumigation of 62 days with MS (93.5 mL m⁻²) and MeBr (48.9 g m⁻²) effectively controlled the Pythium spp. and Rhizoctonia solani populations when the average high temperature at a soil depth of 10.2 cm during application was 18 °C and when soil samples were examined 15 days after polyethylene removal in tobacco and pepper. In 1995-1996, a trial conducted at the University of Georgia in the USA by Csinos et al. (2000) obtained the same effect for MS (34.9 L m⁻²) and MeBr (65 g m⁻²) on 2 soilborne fungi after 26 days of fumigation under a polyethylene film at soil temperatures ranging from 11.1 to 23.3 °C. In our experiment, exposure time to the fumigant was shorter and the soil temperature was lower. We also observed that MS (100 mL m⁻²) effectively controlled damping-off in 2006 when the timing of the


<table>
<thead>
<tr>
<th>Treatments*</th>
<th>Amaranthus spp.**</th>
<th>Portulaca oleracea**</th>
<th>Chenopodium album**</th>
<th>Polygonum aviculare**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. m⁻²</td>
<td>Control (%)</td>
<td>No. m⁻²</td>
<td>Control (%)</td>
</tr>
<tr>
<td>Untreated control</td>
<td>196.42 a</td>
<td>-</td>
<td>8.17 a</td>
<td>-</td>
</tr>
<tr>
<td>MeBr (90 g m⁻²)</td>
<td>0.33 b</td>
<td>99.8</td>
<td>0.00 b</td>
<td>100</td>
</tr>
<tr>
<td>MS (100 mL m⁻²)</td>
<td>0.00 b</td>
<td>100.0</td>
<td>0.00 b</td>
<td>100</td>
</tr>
<tr>
<td>MS (50 mL m⁻²)</td>
<td>0.42 b</td>
<td>99.8</td>
<td>0.00 b</td>
<td>100</td>
</tr>
<tr>
<td>D (50 g m⁻²)</td>
<td>0.25 b</td>
<td>99.8</td>
<td>0.00 b</td>
<td>100</td>
</tr>
<tr>
<td>D (25 g m⁻²)</td>
<td>5.58 b</td>
<td>97.2</td>
<td>0.00 b</td>
<td>100</td>
</tr>
</tbody>
</table>

*MeBr: Methyl bromide; MS: Metam sodium; D: Dazomet  
**Means in columns followed by the same letter are not significantly different (P ≤ 0.05).


<table>
<thead>
<tr>
<th>Treatments*</th>
<th>Plant height (cm)**</th>
<th>Fresh weight (g)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2005</td>
<td>2006</td>
</tr>
<tr>
<td>Untreated control</td>
<td>12.5 c</td>
<td>15.8 c</td>
</tr>
<tr>
<td>MeBr (90 g m⁻²)</td>
<td>20.8 a</td>
<td>18.7 ab</td>
</tr>
<tr>
<td>MS (100 mL m⁻²)</td>
<td>16.6 b</td>
<td>19.7 a</td>
</tr>
<tr>
<td>MS (50 mL m⁻²)</td>
<td>-</td>
<td>19.6 a</td>
</tr>
<tr>
<td>D (50 g m⁻²)</td>
<td>18.3 ab</td>
<td>20.0 a</td>
</tr>
<tr>
<td>D (25 g m⁻²)</td>
<td>-</td>
<td>16.4 bc</td>
</tr>
</tbody>
</table>

*MeBr: Methyl bromide; MS: Metam sodium; D: Dazomet  
**Means in columns followed by the same letter are not significantly different (P ≤ 0.05).
application (intervals between fumigation and planting) was 10 days shorter than in the previous year. Depending on the fumigant, the interval between treatment and planting for maximum suppression of fungal pathogens can vary with the species evaluated under different soil temperatures and moisture conditions (Seebold and Csinos 2001). Recently, Desaeger et al. (2008) proved that propagules of *R. solani* were more effectively killed following shorter intervals between fumigation and planting (7 days) than after long intervals (>14 days) in trials in the spring of 2002.

Our 2-year study suggested that D (50 g m⁻²) and MS (100 mL m⁻²) effectively controlled all weeds (though *Urtica urens* L. and *Chenopodium murale* L. were less controlled by D in 2005) appearing in plots after 7 or 12 days of fumigation time under low-temperature conditions (Figure 1 and Table 3). In comparison with MeBr in the 2006 trials, reduced doses of the same fumigants were also found to be very effective against *Amaranthus* spp., *Portulaca oleracea* L., *Chenopodium album* L., *Polygonum aviculare* L., and *Sonchus* spp. (Table 4). Similar results were obtained with MS (93.5 mL m⁻²) and D (38.7 g m⁻²) for different species of weeds in tobacco and pepper seedbeds (Csinos et al. 1997). However, Unruh et al. (2002) reported that D (39.2 g m⁻²) and MS (74.8 mL m⁻²) provided 70% and 7% control of *Amaranthus retroflexus* L. in common turfgrass, respectively, after a 3-day fumigation period without a plastic tarp in Florida, USA, in August 1998. Benlioğlu et al. (2002) demonstrated that D (50 g m⁻²) and MS (100 mL m⁻²) effectively controlled *Portulaca oleracea*, *Poa annua*, and *Amaranthus retroflexus* (except *Conyza canadensis* and *Cyperus rotundus*) after a 7-day fumigation period under a plastic tarp on raised beds of strawberry in Turkey in August 2001.

All treatments except for the half-dose of D increased tobacco seedling height and fresh weight when compared to the untreated control. Comparable positive findings for plant height were obtained from MeBr-, MS-, and D-fumigated soil beds in the southeastern USA (Csinos et al. 1997, 2000).

Findings from the first research on MeBr alternatives in tobacco transplant production in Turkey confirm that MS (100 mL m⁻²) and D (50 g m⁻²) (as opposed to weed control alone) can be a fumigant alternative to MeBr. However, longer plant-back periods than those implemented for MeBr accelerated biodegradation of MITC after repeated application (Wharton and Matthiessen 2000; DiPrimo et al. 2001), especially under cool conditions (Porter et al. 2000; Ajwa et al. 2001). These data have led to considerable concern about the acceptance of these products as alternatives to MeBr.

A nonfumigant alternative known as the floatation tray method is now used to produce tobacco seedlings in many countries, such as Australia, Argentina, Brazil, China, Cuba, Kenya, Macedonia, Senegal, Spain, the USA, and Zimbabwe, at both large and small production levels (MBTOC 2002). However, this method has not been used in Turkey and should be considered as a practical alternative. Our studies on it are currently underway and aim to offer a better and cost effective alternative for preplant fumigation in tobacco seedling production.

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**References**


