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RESEARCH ARTICLE

INFLUENCE OF FUNGICIDES ON POST EMERGENCE OF DAMPING-OFF IN TOBACCO (*NICOTIANATOBACUM L.*) NURSERY

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ABSTRACT

Present investigations were carried out to test aset of four fungicides i.e. Ridomil-G, Dithane M-45, Daconil 75 WP and Acrobat MZ and their different rates were tested for the control of post emergence damping off disease on tobacco seedlings by pre-sowing application of these fungicide sat the Tobacco Research Station, Khanghari, Mardan during the year 2013-14. Two varieties of tobacco i.e. Speight G-28 and TM 2008 were used for this study. All fungicide and their rates were proved effective in the controlling of the disease as compared to the control treatments, while the least significant difference was P ≤0.05.In Speight G-28 variety, Ridomil-G was found effective in the control of post-emergence damping off disease both at10 mL and 5 mL concentration, while Dithane M-45 was effective at 15 mL concentration, Daconil was found to be effective at 10 mL concentration and Acrobat MZ was effective at 5 mL concentration. In TM-2008 variety, Dithane M-45 was effective at 15 mL concentration, Ridomil-G at 5 Ml concentration while, Acrobat-MZ and Daconil fungicides were effective at 10 mL concentration.

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INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is a broad leaved perennial herbaceous plant of family Solanaceae. The word *Nicotiana* is in the honor of Jean Nicot. The common names of the plants are Petume, Petina, Pitura, Etum, Tabaco, Tobacco and Tambako locally (Kamal Kishore 2014).It is a cash crop and is consumed in various ways, i.e. bheedi, chewing tobacco, cigars, cigarettes, creamy snuffs, snuffs, snus, dipping tobaccos, gutkas, kreteks, pipe smoking, hookahs, tropical tobacco paste, tobacco water, etc (http://en.wikipedia.org/wiki/Tobacco).The tobacco production play important role in the economy of a country. The production of the tobacco leaved increased in developing countries by 128%.Since 2005 every year 6.7 million tons of tobaccos are produced throughout the world (Workman, 2006). The tobacco is severely attacked by various pathogens including mycoplasmas, virioids, virus, bacteria, and fungi. The most common diseases of tobacco are angular leaf spot, crown gall, wisconsin bacterial leaf spot, anthracnose, black mildew, black shank, yellow dwarf, big bud, stubby root, and the most important and serious threat is damping-off of seedlings of tobacco at nursery stage (Nyvall, 1979). Damping off is applied to the sudden collapse of seedlings, which are affected at the base of the stem and fall over from weakening of the tissues at this point. Mainly there are two types of damping-off. In pre-emergence damping-off the seed infect as soon as moisture penetrates the seed coat or a bit later as the radicals begin extend, all of which rot immediately under the soil surface. In Post emergence damping-off the cotyledons break the soil surface only to wither or die or healthy looking seedlings may fall over. Infection results in lesions at or below the soil. The various species causing damping off i.e. *Rhizocontia* sp, *Phytium*sp, *Thielaviopsisbasicola*, *Phytophthora*sp, and other miscellaneous fungi include *Sclerotiurolfsii*, *Macrophominaphasoeli, Fusarium* etc (Georgakopoulos *et al.,* 2002). Fungicides are Chemical substances that destroy or inhibit the growth of fungi are termed as fungicides. The purpose of selection of fungicides as control agent is because the disease is caused by fungi. The Bordeaux mixture fungicide (copper sulfate and lime), discovered accidentally in 1882, was used successfully against vine downy mildew; this compound is still employed to combat it and potato blight and many other diseases (Wang *et al.,* 2011).

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A good fungicide should be toxic to the parasite and not to the host. It should be reasonably easy to prepare and not too expensive .it should be capable of even distribution from the spraying or dusting. It should have good sticking properties that it is not removed off easily from the surface of the host (Knight *et al.,* 1997). Metalaxyl-M is a systemic fungicide which is rapidly taken up by the green plant parts (within 30 minutes), and transported upwards (acropetally) in the cell sand is distributed in the leaves. It provides control of fungi within the plant, inhibiting fungal growth and reproduction, and protects new growth produced after application. Mancozeb is a protective residual fungicide, which provides a protective film over the plant surfaces, and inhibits germination of the spores. By combining these two ingredients, Ridomil Gold provides excellent disease control from within the plant and externally (Rebollar-Alviter *et al.,* 2005). Dithane M-45 is a contact fungicide with protecting activity. It forms a slight film on the surface of the plant and gradually releases several Degradation compounds, which disrupt the respiratory activity of the Target fungi at several points of metabolic pathway. It is active against spore germination (Dow, Agrosciences). Acrobat WPcontains dimethomorph which interrupts cell wall formation and inhibits Spore growth, attachment and penetration, with some stages being inhibited at concentrations of less than 0.25 ppm. An application of dimethomorph prior to the formation of sporangia also results in complete inhibition of sporulation (antisporulant), stopping the spread of disease. Dimethomorph penetrates leaves and stems (translaminar uptake and rainfast). Dimethomorph moves acropetally (xylemmobile) inside the leaf and stems (Kortekamp *et al.,* 2003). Daconilis a contact fungicide exhibits a multi-site mode of action, which can delay or prevent the development of resistance to single-site fungicides by attacking the disease-causing pathogen at several biochemical sites. The built-in surfactant in Daconil provides superior sticking power for extended coverage on the crown and sheath of grass blades for unsurpassed protection even after heavy rains or watering. The surfactant provides residual control for 14 to 21 days when used at recommended rates (Waggoner, 2011).Therefore, the following study was conducted to control the loss of Post emergence damping off disease and to find out the most suitable and effective fungicides for the control of this disease by pre-sowing application of fungicides.

MATERIALS AND METHODS

Trial site and conditions

A set of four fungicides, Ridomil –G, Dithane M-45, Daconil 75 WP and Acrobat MZ was tested for the control of post-emergence damping off disease on tobacco nursery by pre sowing application of these fungicides. The beds were prepared in the end of November following Sowing of seeds in December. For Protection Against heavy rains and cold temperature the beds were protected by plastic to provide a constant temperature for their germination and growth, seed sheds containing a double layer of plastic film reach the highest temperatures than single layer of plastic (Porras-Soriano, 2009).

Fig.1. Beds of seedlings of tobacco protected in plastic film layer

Experimental design

Tobacco seeds were sown in raised beds with in four rows and 5-6 cm above, for every concentration of fungicide there were four equal replicas of $1m^2$ each. Every row has eight plots in which in the first four plots one variety Speight G-28 was sown followed by TM -2008 variety in the remaining four blocks. Every variety is applied with different concentrations of fungicides e.g. 5 mL, 10 mL, 15 mL, with Spray pump for uniform applicationand control treatment in which there is no application of fungicides. The different fungicides concentrations were mixed with water 1000 mL in each block so, that what concentration is most effective in controlling disease incidence. With the help of electric balance different concentration of fungicides were mixed with 1000 mL of water, followed by spraying with a small hand operating pressure sprayer and a uniform coverage to the sub plots. The size of the plot for each treatment was kept 1 m^2. The fungicides were applied pre sowing of seeds.

Table 1. Different fungicides and their rates of application on 2-varieties of tobacco (Speight G-28 and TM 2008)

S. No	Fungicides	Dose
1	Dithane M-45	5 g / 1000 mL water/ m^2
	Dithane M-45	10 g / 1000 mL water/ m^2
	Dithane M-45	15 g / 1000 mL water/ m^2
	Dithane M-45	control
2	Daconil WP	5 g / 1000 mL water/ m^2
	Daconil WP	15 g / 1000 mL water/ m^2
	Daconil WP	control
3	Acrobat MZ	5 g / 1000 mL water/ m^2
	Acrobat MZ	10 g / 1000 mL water/ m^2
	Acrobat MZ	15 g / 1000 mL water/ m^2
	Acrobat MZ	control
4	Ridomil -G	5 g / 1000 mL water/ m^2
	Ridomil -G	10 g / 1000 mL water/ m^2
	Ridomil -G	15 g / 1000 mL water/ m^2
	Ridomil -G	control

For disease patches in the and the places here there was no seedlings in the quadrate were measured for determining the effectiveness of the fungicides, while the weakening of the stem near the soil surface, poor plant stand, diseased area in the form of patches, the pale color of seedlings and stunted growth were taken as criteria for recording observations. The affected seedlings were studied in the laboratory of Pakistan Tobacco Board for the isolation and identification of casual organisms. The experiments were conducted on a naturally infested field (Hussain *et al.,* 1980).

Plant Density and Diseases Intensity Estimation

Plants density (Plants Per quadrate).The data were observed randomly, (Bookhout, 1996). The sizes of the quadrate were 4×4 cm^2. By throwing quadrate in the plots and counting the numbers of healthy plants and diseased plants, and repeating this five times in one plot (Shinwari *et al.,* 2000). And then summing up the data of the five quadrates, so for one plot total and diseased plants were obtained, and this is applied for all plots and concentrations. The data were observed after every six days interval. The weeds were removed from beds after each 20 days interval. The results were analyzed statistically by SPSS Version 4.0

RESULTS

A set of four fungicides, Ridomil-G, Dithane M-45, Daconil 75 WP and Acrobat MZ and their different rates were tested for the control of Post emergence damping off disease on tobacco seedlings, by pre sowing application of these fungicides at the Tobacco Research Station, Khanghari, Mardan, the results showed that all fungicides were effective in the control of post emergence damping off disease as compared to control.

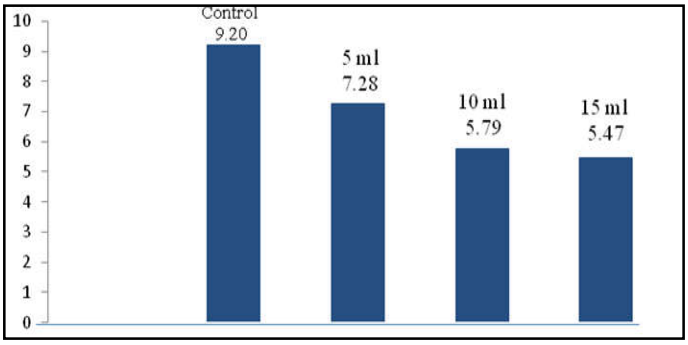

Figure 1. Intra varietal comparison of fungicide Dithane M-45 in variety speight G-28

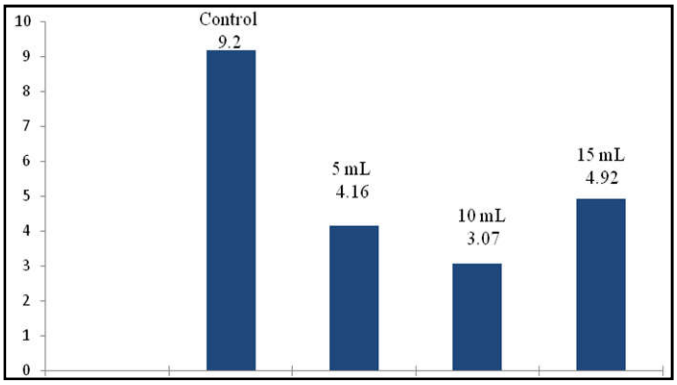

Figure 2. Intra varietal comparison of fungicide Ridomil-G in variety speight G-28

Comparison of different fungicidal concentration for the control of post emergence damping off disease in Speight G-28 variety

Dithane M-45 had given good control of post-emergence damping off disease as compared to control. Dithane M-45 was most effective at 15 mL concentration followed by 10 mL concentration, while Dithane M-45 in 5 mL concentration gives the lowest disease control among the same fungicidal treatments (Fig. 1). Fungicide Ridomil-G had given good control of post-emergence damping off disease as compared to control treatment. Ridomil-G was most effective at 10 mL concentration followed by 5 mL concentration while, at 15 mL concentration it was least effective against disease (Fig. 2). Arobat MZ shown a relatively high proportion of damping off disease as compared with control treatment. Acrobat MZ was best at 5 mL concentration in the control of damping off disease, followed by 10 mL concentration while it was ineffective at 15 mL concentration (Fig. 3). Daconil fungicide was also effective in the control of damping off disease as compared to control.It was most effective at 10 mL concentration, followed by 5 mL concentration while, at 15 mL concentration it was least effective (Fig. 4)

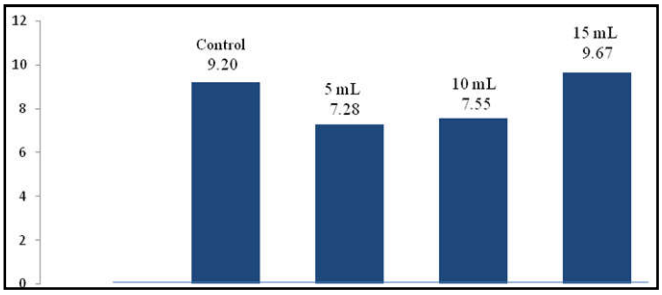

Figure 3. Intra varietal comparison of fungicide Acrobat MZ in variety speight G-28

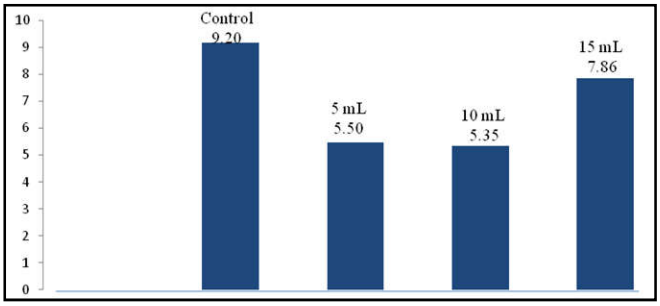

Figure 4. Intra varietal comparison of fungicide Daconil in variety speight G-28

Comparison of different fungicidal concentration for the control of post emergence damping off disease in TM-2008 variety

Dithane showed good control of post-emergence damping off disease as compared to control treatment. Dithane was best at 15 mL concentration in controlling disease followed by 10 mL concentration, while it was least effective at 5 mL concentration (Fig. 5). Ridomil-G had shown good control of post-emergence damping off as compared to control. This fungicide was most effective at 5 mL concentration followed by 10 mL concentration, while the lowest control of disease was at 15 mL concentration (Fig. 6). Acrobat MZ was effective at 10 mL concentration followed by 5 mL concentration, while at 15 mL concentration it was found ineffective (Fig. 7). Daconil had shown good control at 10 mL concentration followed by 5 mL concentration, while it was ineffective at 15 mL concentration (Fig. 8).

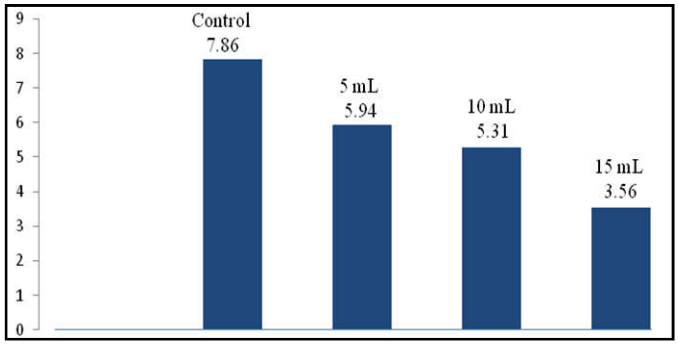

Figure 5. Intra varietal comparison of fungicide Dithane M-45 in variety TM 2008

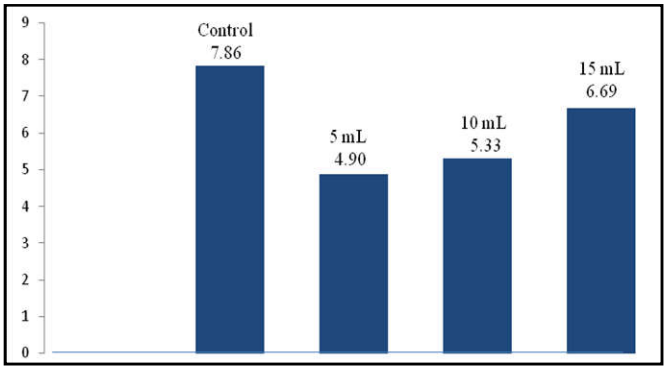

Figure 6. Intra varietal comparison of fungicide Ridomil G in variety TM 2008

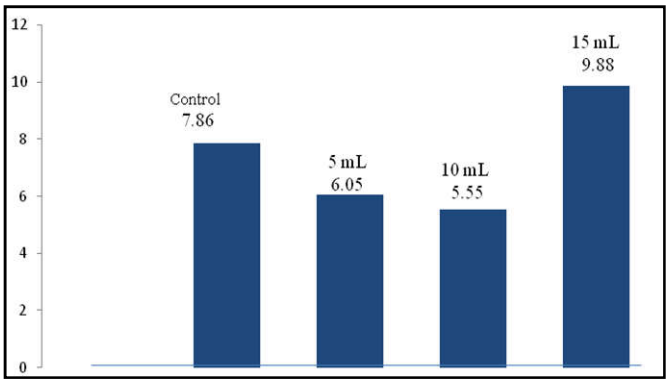

Figure 7. Intra varietal comparison of fungicide Acrobat MZ in variety TM 2008

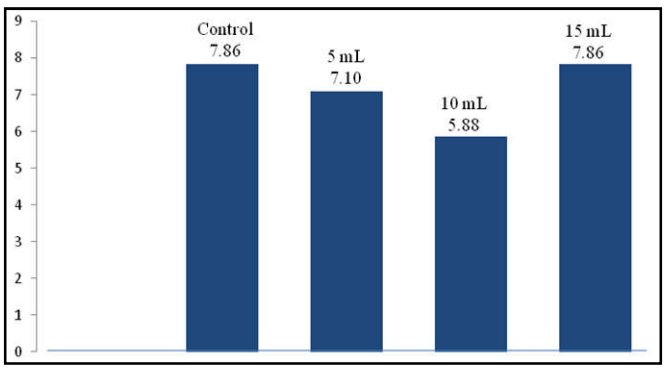

Figure 8. Intra varietal comparison of fungicide Daconil in variety TM 2008

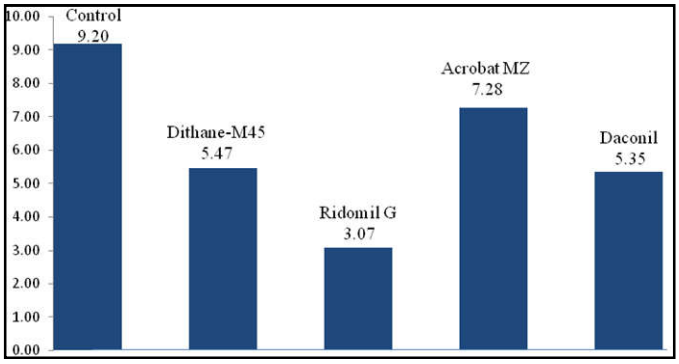

Figure 9. Fungicides concentrations which give highest control among all rates andcontrol treatment

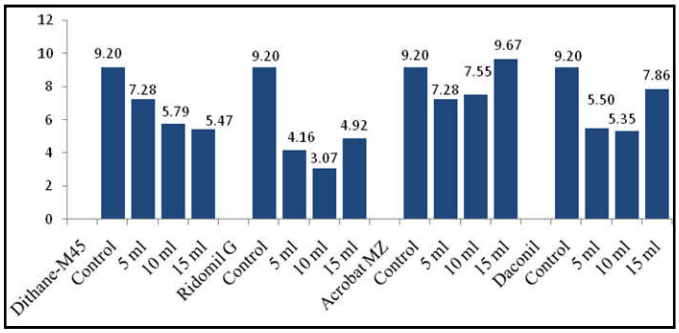

Figure 10. Different concentration of fungicides and control treatment in SpeightG-28 variety of tobacco

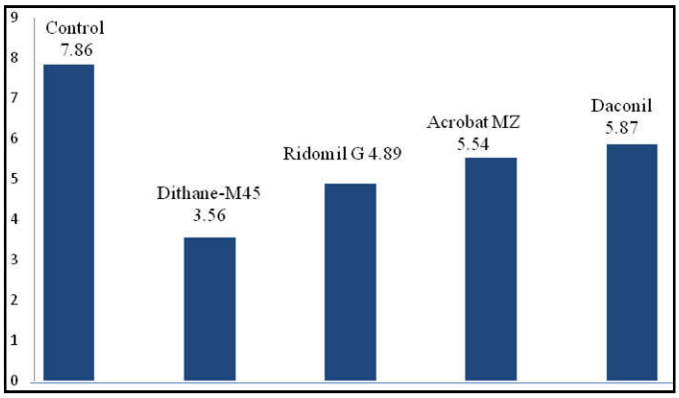

Figure 11. Fungicides concentration which gives highest control among all ratesand control treatment

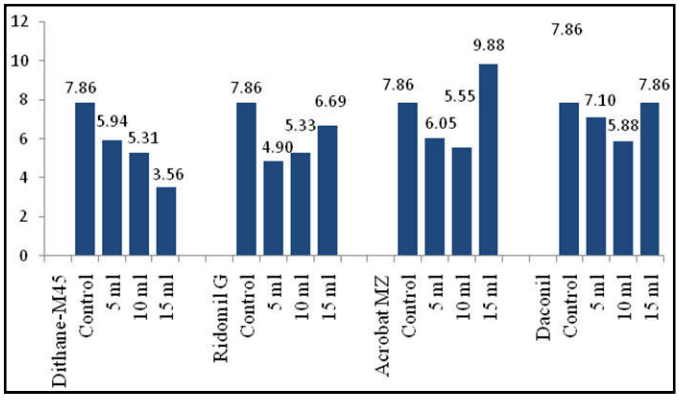

Figure 12. Different concentration of fungicides and control treatment inTM -2008 variety of tobacco

Comparisons of all fungicides and their concentration with control treatment in Speight G-28 variety

Ridomil G had shown best control of post-emergence damping off disease in Speight G-28 variety of tobacco at 10 mL concentration among all the fungicides, followed by 5 mL concentration, while the Dithane M-45 was most effective against damping off at 15 mL concentration however, Daconil was ranked 3rd in controlling disease and was found most effective at 10 mL concentration. Among the four used fungicides, Acrobat MZ was least effective against damping off of tobacco in Speight G-28 variety (Fig. 9). The overall result showed that at 15 mL concentration Acrobat MZ and Daconil was ineffective while, Dithane M-45 at 5 mL concentration (Fig. 10).

Comparisons of all fungicides and their concentration with control treatment in variety TM -2008 of tobacco

All the fungicides applied for the post emergence damping off disease had shown control of disease as compared to control treatment in variety TM -2008. Dithane M-45 was most effectiveagainst post-emergence damping off disease among all the fungicides at 15 mL concentration, followed by Ridomil G at 5 mL concentration while Acrobat MZ rank 3rd in the control of Post emergence damping off disease at 10 mL concentration. Daconil fungicides were found effective at 10 mL concentration, the lowest control was observed in Daconil fungicideand the least significant difference was $P \leq 0.05$ (Fig. 11). The result revealed that the fungicides Acrobat MZ, Daconil and Ridomil at 15 mL concentration did not give any significant results as compared to control treatment (Fig. 12).

DISCUSSION

Damping off is applied to the sudden collapse of seedlings, which are affected at the base of the stem and fall over from weakening of the tissues at this point, the disease areas are more or less circular and all seedlings are killed as the patches enlarges. A brown, watery soft-rot develops, the hypocotyls are girdled and the seedlings toppled to lie prostrate on the ground. Mainly damping off attacked seedlings at two stages i.e. pre and post-emergence stage. The present study was conducted to develop newer methods for better control of tobacco nursery diseases by suitable fungicides and also their most active concentration. Our result of different fungicides in the control of Post emergence damping off disease revealed that all fungicides significantly controlled the disease as compared to the control treatments. Similar results have also been reported by many workers including Liu and Chen (1988); Anonymous (1973); Hussain *et al.* (1980); Hussain and Shah (1993) and Wadood *et al.* (2007-08). RidomilG fungicide was most effective at 10mL concentration among all the fungicides and their different concentrations in Speight G-28 variety. While the findings of Hussain and Shah (1993); Wadood *et al.* (2007-08) ranked Ridomil-G fungicide second in its effectiveness as compared to other fungicides. However, GWRDC Final Report, Project No.SAR 99/3 were agreed with our results. Similarly, Koike *et al.* (1992); Raziq *et al.* (2008): Iqbal *et al.* (2003); Mburu Njoroge (1978-79) also agreed with the same results.

Our result revealed that in Speight G-28 variety, fungicide Dithane M-45 was most effective at 15 mL concentration whereas, Daconil and Acrobatfungicides had shown best control of Post emergence damping off disease in Speight G-28 variety at 10 mL concentration. Similar results were presented by Hussain *et al.* (1980); Hussain and Shah (1991) and Hussain and Shah (1993). According to Wadood *et al.* (2007-08) Dithane M-45 ranked first in the control of damping off disease.Our result in variety Speight G-28, following rates of fungicide are not showing good control of post emergence damping disease e.g. in the concentration 15mL ,Acrobat MZ and Daconil and Dithane M-45 at 5mL concentration , showing no significant results Iqbal *et al.* (2003) The result agrees with that of the findings of Bugspray. Our results in variety TM-2008, Fungicide Dithane M-45 was the most effective among all the fungicides at 15 mL concentration, the result also agrees with the findings of Annonymous (1973); Hussain *et al.* (1980); Hussain and Shah (1991); Hussain and Shah (1993); Wadood *et al.* (2007-08).Our results revealed that in variety TM-2008, fungicide Ridomil-G was most effective in controlling post emergence damping off disease after Dithnae M-45. The Fungicide gives best results at 5 mL concentrations, these result are accordance with that of the findings of Annonymous (1973); Hussain *et al.,* (1980); Hussain and Shah (1991); Hussain and Shah (1993); Raziq *et al* . (2006): Iqbal *et al*. (2003) Wadood *et al.* (2007-08); Koike *et al*. (1992); Raziq *et al*. (2006): Iqbal *et al*. (2003) and Mburu Njoroge (1978-79).Our results on post emergence damping off control in variety TM-2008, fungicides Acrobat MZ at 10 mL concentration after Dithane M-45, and Ridomil-G was effective these results also confirms with those off Anonymous (1973); Hussain *et al.* (1980); Hussain and Shah (1991); Hussain and Shah (1993); Wadood *et al.* (2007-08).Our results had shown that fungicides Daconil was effective in the control of post-emergence damping off in variety TM-2008but it was least effective among all the fungicides. Similar results were presented by Wadood *et al.* (2007-08); Hussain and Shah (1991); Hussain *et al.* (1980) andHussain and Shah (1993). It was also revealed from the present study that at 15 mL concentration, fungicides Acrobat MZ, Daconil and Ridomil did not give any significant results in variety TM-2008as compared with the control treatment as also shwn by Raziq *et al.* (2006) and Iqbal *et al*. (2003).

Conclusion and recommendations

It was concluded from the present work that fungicide in the following order shown the effectiveness against damping off at different concentrations on both tobacco varieties, in variety speight G-28 fungicide Ridomil-G was most effective both at 10mL and 5mL concentration. Whereas, Dihtane M-45 at 15 mL, Daconil at 10 mL and Acobat MZ at 5 mL concentrations. In variety TM-2008; Dihtane M-45 was most effective at 15 mL concentration, Ridomil-G at 5 mL, while Acrobat MZ and Daconil at 10 mL concentrations against damping off. It is recommended from the present study that further research is desired to evaluate selected fungicides and their different concentration in the control of post emergence damping off disease of tobacco and similar research should be carried out to explore the effectiveness of other fungicides against damping off of tobacco and in other crop plants.

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