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Effects of IBA and NAA on Rooting Male sterile and Hermaphroditic Types of Three Iranian Thymus Species

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ABSTRACT

The rooting performance of cuttings from three endemic thyme species, *Thymus daenensis*, *T. migricus*, and *T. pubescens*, was evaluated using Indole-3-butyric acid (IBA) and Naphthaleneacetic acid (NAA) solutions at four concentrations (0, 200, 500, and 1000 ppm). The study was conducted in a factorial randomized block design with three replicates. Key parameters assessed included rooting percentage, number of roots per cutting, longest root length, lateral shoot length, number of lateral shoots per cutting, and days to the first lateral shoots. Rooting percentage was generally higher with IBA treatment compared to NAA. All other characteristics varied depending on the species and the concentration of the rooting solutions. The highest number of roots per cutting (4) and the longest root length (9.66 cm) were observed in *T. daenensis* Male Fertile. This species also produced the longest lateral shoots (10 cm). The maximum number of lateral shoots (9.66) was recorded in both Male Sterile and Male Fertile forms of *T. daenensis*. Additionally, *T. pubescens* exhibited faster lateral shoot emergence compared to T*. migricus* and *T. daenensis*. The results suggest that while both NAA and IBA have similar effects on rooting, concentrations of 200 and 500 ppm are optimal for thyme species. The study also concluded that male sterile plants outperformed male fertile plants in terms of rooting rate and root production. These findings are valuable for breeding programs and developing plant clones.

Abbreviations: Indole-3-butyric acid (IBA), Male sterile (MS), Hermaphrodite/ male fertile (MF), 1-Naphthaleneacetic acid (NAA), *T. daenensis* Male Sterile (dMS), *T. daenensis* Male Fertil (dMF), *T.migricus* Male Sterile (mMS), *T.migricus* Male Fertile (mMF), *T. pubescens* Male Sterile (pMS), *T. pubescens* Male Fertile (pMF)

Introduction

Medicinal plants have been used globally as medicines, flavoring agents, and preservatives (Araghi et al., 2019). The secondary metabolites found in these plants and mushrooms are utilized in various fields, including medicine and the food industry (Azizi et al., 2012). The primary goal of medicinal plant production is the extraction and processing of these secondary metabolites (Azizi et al., 2010). Researchers often investigate the factors influencing these metabolites, such as environmental conditions, genetics, fertilization, domestication, and post-harvest activities (Azizi et al., 2009; Ebadi et al., 2017; Heidari et al., 2014; Shahhoseini et al., 2020).

Genetic variation serves as a key source for plant breeding through techniques like selection, hybridization, and mutation. Garden thyme (*Thymus vulgaris* L.) has a gynodioecious reproductive system, where populations exhibit two flower types: hermaphrodites and females (Mohammadi et al., 2021). This dimorphism often results from interactions between cytoplasmic male sterility (CMS) genes and nuclear restorer of male fertility genes (Mollion et al., 2017). CMS genes are particularly valuable in breeding programs for hybridization (Mohammadi et al., 2020).

The global use of aromatic and medicinal plants, such as thyme (*Thymus* spp.), is increasing (Araghi et al., 2019). Belonging to the Lamiaceae family, thyme is a rich source of phytochemicals and bioactive compounds. Gynodioecious *Thymus* species are known to have a high (>50%) and variable frequency of female plants in their populations (Manicacci et al., 1998; Stahl-Biskup and Sáez, 2002). The *Thymus* genus includes approximately 267 species worldwide (POWO, 2023), with 18 species growing in Iran. Some of these species include *T. migricus* Klokov & Desj.-Shost, *T. daenensis* Celak (Avishan-e-denaee), and *T. pubescens* Boiss. & Kotschy ex Celak (Jamzad, 2013). Four of these species are endemic, with *T. daenensis* being one of Iran's medicinal plants (Mozaffarian, 1996; Shayganfar et al., 2018).

The essential oil yield in various populations of *T. daenensis* ranges between 1.87% and 3.83% (Rustaiee et al., 2010). Several studies highlight the high levels of thymol in *T. daenensis*, with thymol values of 68.5% and 66.3%, followed by carvacrol at 13.6% and 14.2% in hermaphrodite and female plants, respectively (Mohammadi et al., 2021). *Thymus migricus*, a perennial herb with pinkish flowers, grows in northwest Iran, where locals use it as a flavoring agent for herbal tea. The essential oil yield for *T. migricus* is reported to be 1.5% based on dry weight (Yavari et al., 2010). Meanwhile, *Thymus pubescens* grows widely in regions like Azerbaijan, Isfahan, Hamedan, Mazandaran, Qazvin, and Tehran provinces of Iran (Mahmoudi et al., 2008), with essential oil yields of 1.47% (v/w) in hermaphrodite plants and 1.02% in female plants. Carvacrol is the main component in the essential oils of both types (60.8% in hermaphrodites and 61.3% in females) (Mohammadi et al., 2021).

Considering the distinct reproductive traits of hermaphrodite and female thyme species, studying their reproduction methods can provide valuable insights for breeding programs. Vegetative propagation is essential for thyme breeding through hybridization, as it ensures that propagated plants maintain the same genotype as the parent plant, preserving the characteristics of the mother plant (Ingram and Yeager, 1986; Ruchala et al., 2002). Research on thyme species shows that Indole-3-butyric acid (IBA) significantly enhances the rooting of cuttings in various species, including *T. satureioides* (Karimi et al., 2014), *T. capitatus*, *T. serpyllum* (Iapichino et al., 2006), *T. vulgaris* (Chandregowda and Shiva, 2008), and *T. kotschyanus* Boiss. (Bahadori and Sharifi Ashorabadi, 2017).

Given the favorable quantitative and qualitative traits of endemic thyme species, they hold significant potential for breeding efforts. The vegetative propagation of male sterile (MS) and male fertile (MF) thyme plants is crucial for breeding programs, enabling the production of clones that retain the traits of the mother plant. This study aims to compare MS and MF thyme plants in terms of rooting ability, assess the impact of different auxin hormones (IBA and NAA) and their concentrations on root formation, and identify the most effective hormone and concentration for each plant type.

Materials and Methods

Plant materials

This study examined three Iranian thyme species, *Thymus daenensis*, *T. migricus*, and *T. pubescens*, along with two flower types from each species: female/male sterile (MS) and hermaphrodite/male fertile (MF). The following codes were used: dMS for *T. daenensis* Male Sterile, dMF for *T. daenensis* Male Fertile, mMS for *T. migricus* Male Sterile, mMF for *T. migricus* Male Fertile, pMS for *T. pubescens* Male Sterile, and pMF for *T. pubescens* Male Fertile.

Stem cuttings were collected from MS and MF plants grown in the horticultural greenhouse at Ferdowsi University of Mashhad, Iran, in November 2023. These cuttings measured 7–10 cm in length and 0.3–0.4 cm in diameter, with 4–5 buds each. They were taken from healthy, well-established, 6-year-old mother plants. To reduce transpiration, up to 50% of the leaves were removed, and the cuttings were washed under running tap water to remove surface contaminants.

Experimental design and hormone treatments

The experiment was conducted in a randomized complete block design (RCBD) with three factors and three replications. The factors included different concentrations of IBA (Indole-3-butyric acid) and NAA (Naphthaleneacetic acid), both at four levels (0, 200, 500, and 1000 ppm), and three thyme species with two flower types (MS and MF), resulting in six treatment groups: dMS, dMF, mMS, mMF, pMS, and pMF.

The cuttings were treated with plant growth regulators using the quick dip method, where they were immersed for 25 sec. After drying for five min, the cuttings were planted in trays containing a mixture of sand and cocopeat (in a 3:1 ratio) with a small amount of perlite (Fig. 1).

Fig. 1. Cuttings (a), soaking cuttings in hormone solution (b), planting cuttings in a tray (c).

To measure the different traits, the cuttings were carefully uprooted from the bed to avoid damaging the roots and were washed with water. The attributes measured included rooting percentages, the number of roots per cutting, the length of the longest root, the length of lateral shoots, the number of lateral shoots per cutting, and the number of days taken for the first lateral shoot to emerge. Observations varied among different plants. For each species, traits were measured as soon as rooting and shoot growth were visible.

A ruler was used to measure the length of the lateral shoots, while the days taken for the first lateral shoot to emerge were recorded by observing the treated cuttings daily under each treatment for sprouting. The longest root of each rooted cutting was measured from the base to the tip using a measuring scale, and the mean root length was calculated.

Caring for the cuttings

After planting, the cuttings required consistent watering. To minimize water loss through transpiration, high air humidity and soil moisture were maintained by regular spraying.

Statistical analysis

Data analysis was performed using SAS software (version 9.1, SAS Institute, Inc., Cary, NC, USA). Duncan's multiple range test (DMRT) was applied ($P \leq 0.01$) to determine significant differences between plant types, hormone treatments, and concentrations.

Results

Rooting percentage, the number of roots per cutting, the length of the longest root, lateral shoot length, the number of lateral shoots per cutting, and the days to first lateral shoot emergence were significantly influenced by different plant species (P ≤ 0.01) and various concentrations of IBA and NAA (P ≤ 0.05). However, the type of hormone used did not significantly affect any of the traits studied.

Rooting percentages

Effect of NAA treatment

In *Thymus daenensis*, comparisons of mean values of the rooting percentages showed that under NAA treatment, dMS plants had a higher rooting percentage than dMF plants. In contrast, under IBA treatment, dMF plants exhibited a higher rooting percentage than dMS. Overall, the highest rooting percentages for both dMS and dMF (100%) were recorded at a concentration of 200 ppm NAA (Fig. 2a).

In *T. migricus*, the application of hormones generally increased rooting percentages. Rooting percentages were consistently higher in mMS than in mMF. Under NAA treatment, mMS plants treated with 200 ppm NAA achieved the highest rooting percentage (100%) (Fig. 2a). No rooted cuttings were observed at 1000 ppm NAA or in the control group (Fig. 2a).

In *T. pubescens*, rooting percentages were lower compared to the other two species across all IBA and NAA treatments. However, pMS exhibited a significantly higher rooting percentage than pMF. In the NAA treatment, pMS cuttings showed the highest rooting percentage (66.66%) when treated with 500 ppm. No rooted cuttings were observed in the control group or in dMS and dMF cuttings treated with 200 ppm NAA (Fig. 2a).

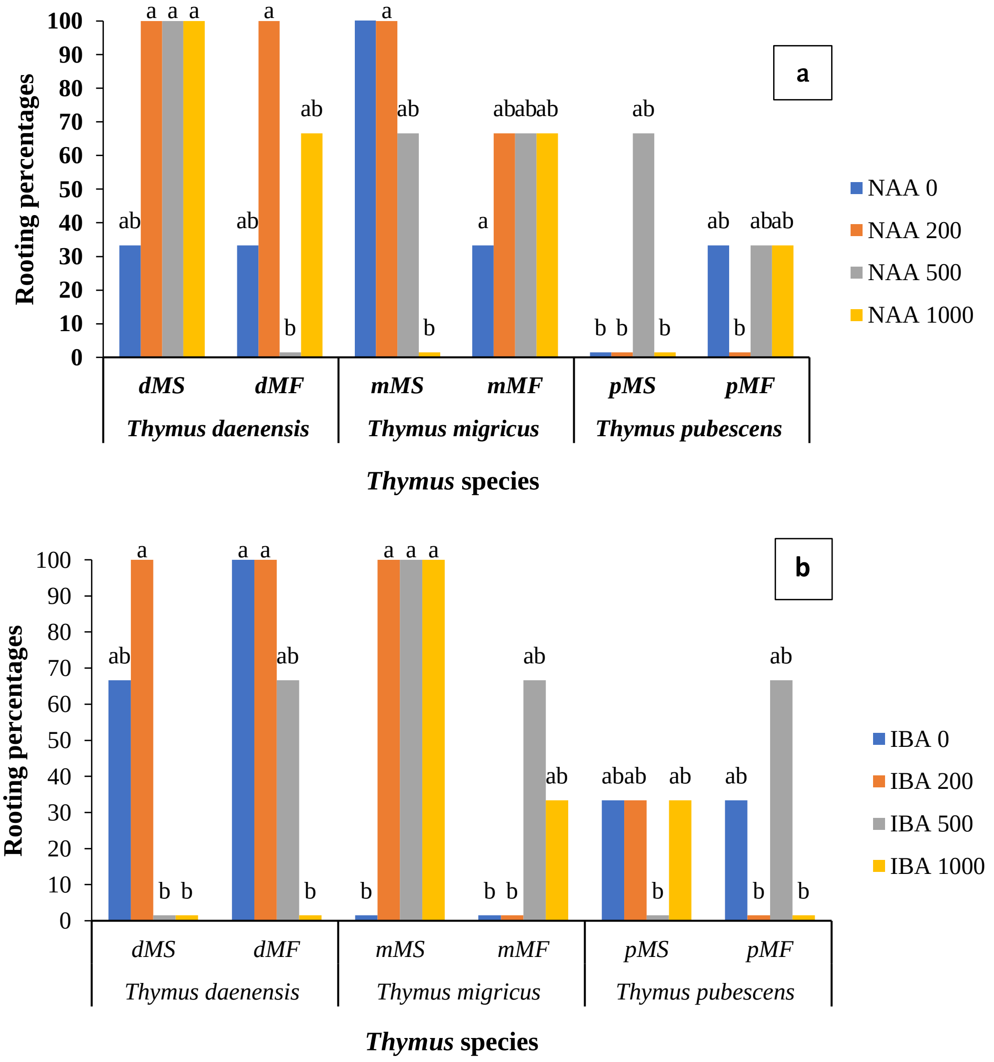

Fig. 2. Effect of different concentrations of NAA (a) and IBA (b) on rooting percentages of different Thymus species (T. daenensis (*Male Sterile)*= dMS*, T. daenensis* (Male Fertile)= dMF, *T.migricus* (Male Sterile*)*= mMS, *T.migricus* (Male Fertile)= mMF*, T. pubescens* (Male Sterile*)*= pMS, *T. pubescens* (Male Fertile)= pMF.

Effect of IBA treatment

The highest rooting percentages for dMS and dMF (100%) were observed at a concentration of 200 ppm IBA. In contrast, no rooted cuttings were obtained from dMS at 500 or 1000 ppm IBA (Fig. 2b). Rooting percentages in *T. migricus* mMS were consistent across different IBA concentrations and reached the maximum (100%) (Fig. 2b), though no rooting was observed at 200 ppm IBA. In general, the application of IBA in mMS resulted in higher rooting percentages than NAA. In mMF, rooting percentages did not significantly differ between IBA and NAA treatments.

For *T. pubescens*, pMS plants exhibited similar rooting percentages across all treatments (33.33%), although no roots were formed in pMS cuttings treated with 200 or 1000 ppm IBA (Fig. 2b). Figure 3 illustrates the root development in cuttings.

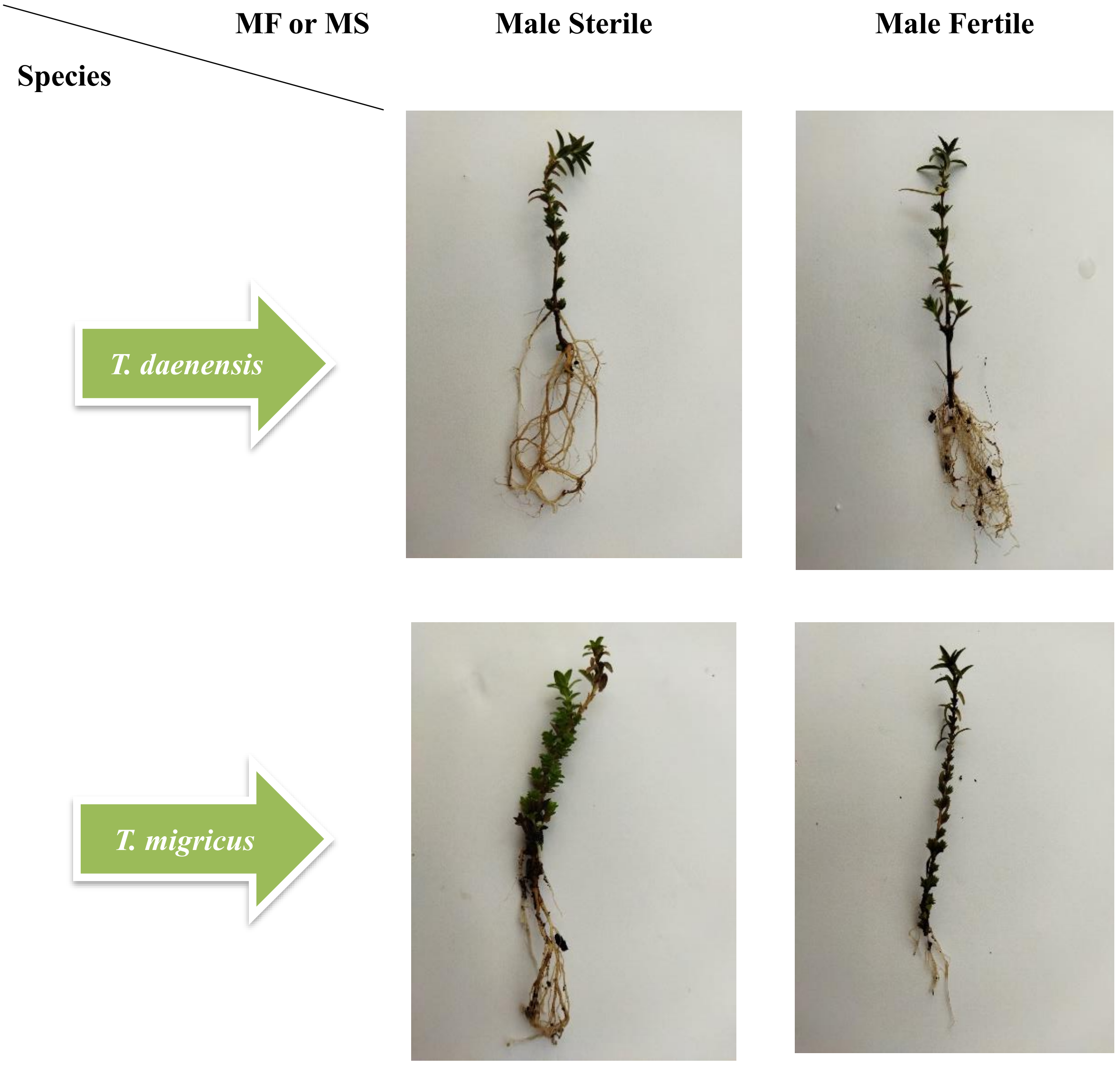

Fig. 3. Rooted cuttings of *T. daenensis*, *T.migricus*, and *T. pubescens*.

Number of roots per cutting

Effect of NAA treatment

The results revealed that the application of 200 ppm NAA in both dMS and dMF produced the highest number of roots per cutting (4) (Fig. 4a). In *T. migricus*, the NAA treatment yielded the highest number of roots in mMS (3.33) when treated with 200 ppm NAA, and in mMF (2.33) when treated with 500 ppm NAA (Fig. 4a). Overall, mMS had more roots than mMF when treated with NAA. For *T. pubescens*, the use of 1000 ppm NAA resulted in the highest number of roots in pMF (1), while no roots were observed in pMS under this treatment (Fig. 4a).

Effect of IBA treatment

In the IBA treatment, the highest number of roots was observed in dMS (4) at a concentration of 200 ppm, and in dMF (3.33) at both 1000 ppm and in the control group (Fig. 4b). Overall, the number of roots in dMF was greater than in dMS. The application of 200 and 1000 ppm IBA produced the highest number of roots (4) in mMS, while mMF exhibited the highest number of roots (2.33) at 500 ppm IBA (Fig. 4b). The results indicated that mMS had more roots than mMF due to the use of the IBA hormone, suggesting that IBA application generally resulted in increased root production in *T. migricus*.

For *T. pubescens*, the highest number of roots (1.33) was recorded in pMS at 500 ppm IBA, while pMF showed a higher root count (4) under the same treatment. No roots were observed in pMF cuttings treated with 200 or 1000 ppm IBA (Fig. 4b). Overall, the application of IBA proved to be more effective than NAA for promoting root development in *T. pubescens*.

The longest root length

Effect of NAA treatment

In general, increasing the concentration of NAA resulted in a decrease in the length of the longest root across all species (Fig. 5a). The longest root lengths recorded were 6 cm in dMS and 7.33 cm in dMF, both treated with 200 ppm NAA (Fig. 5a). In the NAA treatment, mMS exhibited the longest root length of 6.66 cm at 200 ppm, while the lowest root length was observed at 1000 ppm NAA. For mMF, the application of 500 ppm NAA resulted in the longest root length of 3 cm (Fig. 5a). In this study, *T. pubescens* had a shorter root length compared to *T. daenensis* and *T. migricus*. In the NAA treatment, the longest root lengths recorded for pMF and pMS were 2 cm (control treatment) and 1.66 cm (500 ppm NAA), respectively (Fig. 5a).

Effect of IBA treatment

In the IBA treatment, the longest root length for dMS was recorded at 5.66 cm, associated with both the control and 200 ppm IBA, while dMF exhibited the longest root length of 9.66 cm at 1000 ppm IBA (Fig. 5b). For *T. migricus*, the longest root length of 8 cm was observed in mMS at 200 ppm IBA (Fig. 5b). Overall, dMF displayed a greater longest root length than dMS, indicating that IBA application enhanced the root length of *T. daenensis* more effectively than NAA. In mMS, the longest root length of 8 cm was associated with the 200 ppm IBA treatment, while in mMF, the longest root length recorded was 3.33 cm at 500 ppm IBA (Fig. 5b).

In this experiment, mMS consistently exhibited longer root lengths than mMF, and IBA application resulted in longer roots in *T. migricus* compared to NAA application. Notably, *T. pubescens* had shorter root lengths than both *T. daenensis* and *T. migricus*. For the IBA application, the longest root length in pMS was recorded at 2 cm for both the control and 200 ppm treatments, while pMF had its longest root length of 3 cm at the 500 ppm treatment (Fig. 5b). The results indicated that pMF had a longer root length than pMS, and IBA application significantly increased the longest root length compared to NAA in *T. pubescens*.

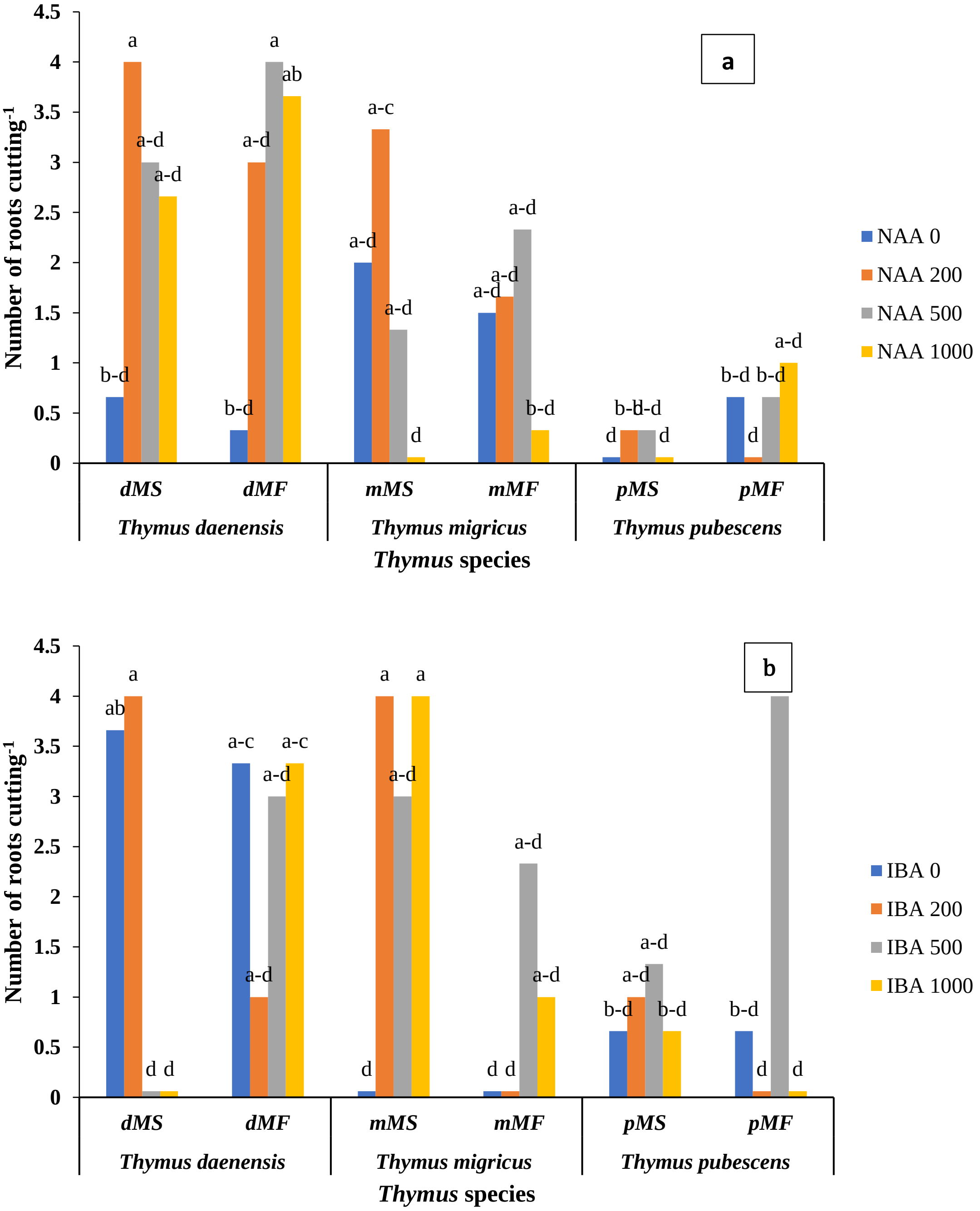

Fig. 4. Effect of different concentrations of NAA (a) and IBA (b) on the number of roots per cutting of different Thymus species (*T. daenensis* (Male Sterile)= dMS, *T. daenensis* (Male Fertile)= dMF, *T.migricus* (Male Sterile)= mMS, *T.migricus* (Male Fertile)= mMF, *T. pubescens* (Male Sterile)= pMS, *T. pubescens* (Male Fertile)= pMF.

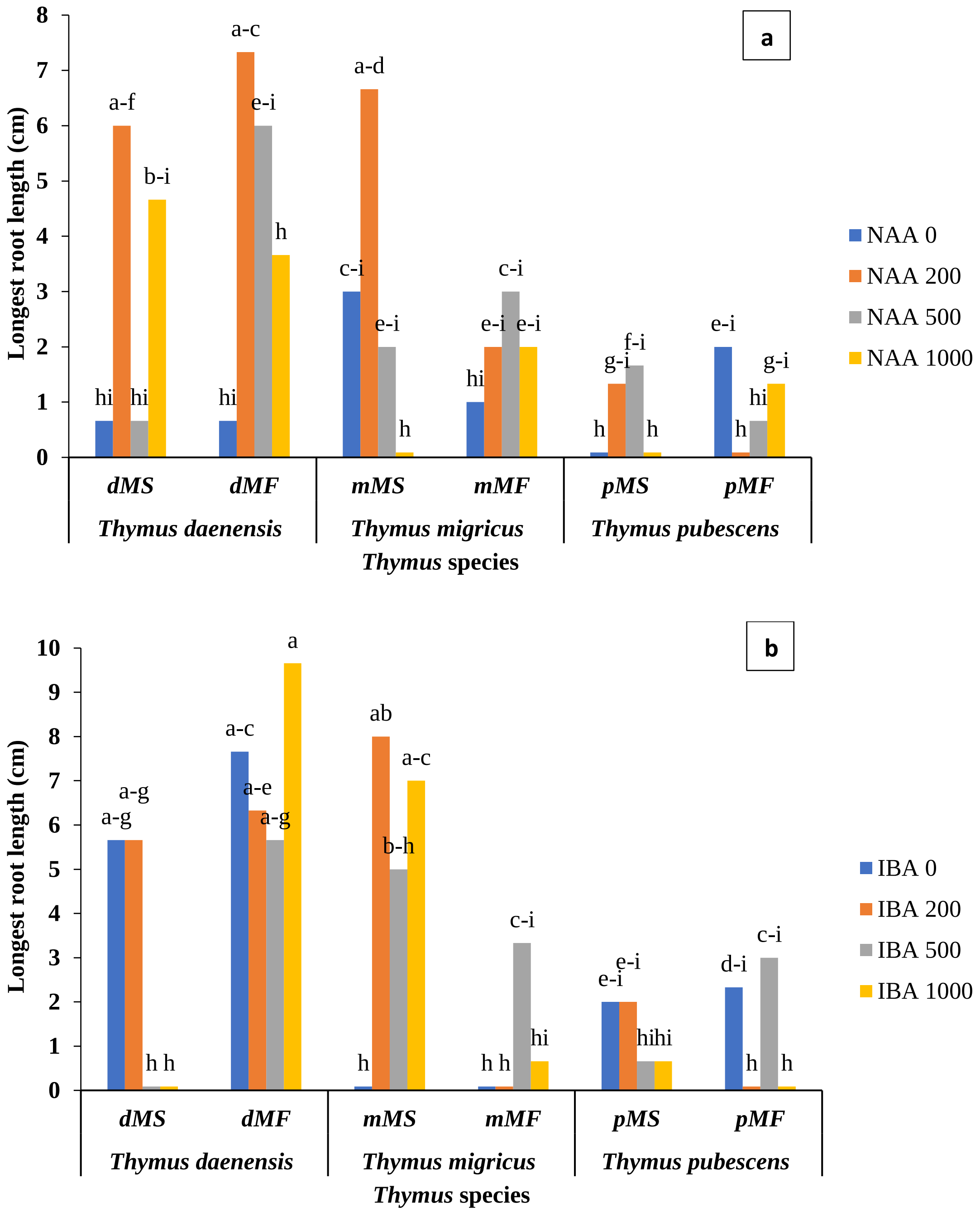

Fig. 5. Effect of different concentrations of NAA (a) and IBA (b) on the longest root length of different Thymus species (*T. daenensis* (Male Sterile)=dMS, *T. daenensis* (Male Fertile)= dMF, *T.migricus* (Male Sterile)=mMS, *T.migricus* (Male Fertile)= mMF, *T. pubescens* (Male Sterile)=pMS, *T. pubescens* (Male Fertile)= pMF.

Number of lateral shoots per cutting

Effect of NAA treatment

The results of this research indicated that the number of lateral shoots per cutting in *T. daenensis* varied with different hormone concentrations. The highest number of lateral shoots, recorded at 9.66, was associated with a 500 ppm NAA treatment in dMS, while dMF exhibited the highest count of 8.66 lateral shoots at 200 ppm NAA (Fig. 6a). The application of NAA notably increased the production of lateral shoots in dMS (Fig. 6a).

In *T. migricus*, the highest number of lateral shoots observed, i.e., 8 in mMS and 6 in mMF, was recorded in the control treatment (Fig. 6a). The results suggested that higher concentrations of

NAA negatively impacted the number of lateral shoots in *T. migricus*. In *T. pubescens*, the number of lateral shoots was lower than in both *T. migricus* and *T. daenensis*. For the NAA application in pMS, lateral shoots appeared only at 200 and 500 ppm concentrations, while pMF showed the highest number of lateral shoots (2.66) in the control and 1000 ppm NAA treatments (Fig. 6a).

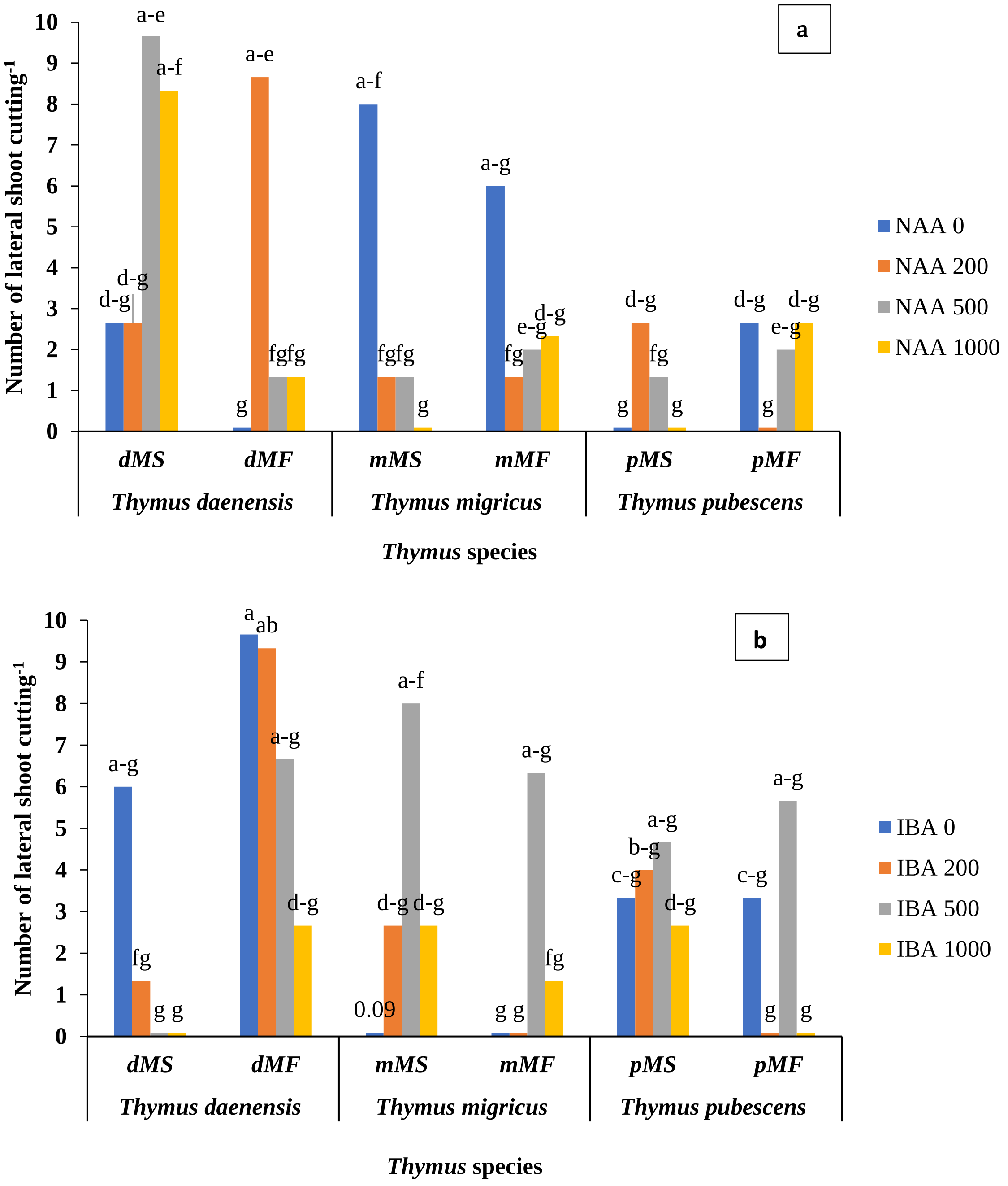

Fig. 6. Effect of different concentrations of NAA (a) and IBA (b) on the number of lateral shoots per cutting of different *Thymus* species (*T. daenensis* (Male Sterile)=dMS, *T. daenensis* (Male Fertile)= dMF, *T.migricus* (Male Sterile)=mMS, *T.migricus* (Male Fertile)= mMF, *T. pubescens* (Male Sterile)=pMS, *T. pubescens* (Male Fertile)= pMF.

Effect of IBA treatment

The application of IBA in the control treatment resulted in the highest number of lateral shoots, with counts of 6 for dMS and 9.66 for dMF, respectively (Fig. 6b). Overall, the application of IBA in dMF led to the production of more lateral shoots. A concentration of 500 ppm IBA yielded the highest number of lateral shoots in *mMS* and *mMF*, with values of 8 and 6.33, respectively (Fig. 6b). However, the results indicated that higher concentrations of IBA in *T. migricus* reduced the number of lateral shoots. For the IBA application test, the highest counts of lateral shoots, i.e., 4.66 in pMS and 5.66 in pMF, were observed at a concentration of 500 ppm (Fig. 6b). In general, the use of IBA promoted greater sprout production in *T. pubescens*.

Lateral shoot length

Effect of NAA treatment

The comparison of mean values indicated that in *T. daenensis*, the maximum lateral shoot length (9.66 and 8.33 cm) was recorded at a concentration of 200 ppm NAA for dMF and dMS, respectively (Fig. 7a). Overall, the results suggested that the application of NAA resulted in greater lateral shoot length in dMF compared to dMS. In the NAA application experiment for *T. migricus,* the maximum lateral shoot lengths (8 and 4.66 cm) were associated with the 200 ppm concentration for mMS and mMF, respectively, while the lowest lateral shoot lengths for both flower types were recorded in the control treatment (Fig. 7a). The findings showed that NAA enhanced lateral shoot length more than IBA in *T. migricus*, with mMS exhibiting longer lateral shoots than mMF. For *T. pubescens*, the length of the lateral shoots was shorter than that observed in *T. daenensis* and *T. migricus*. In the NAA application test, pMS treated with 200 ppm exhibited the longest lateral shoot length (3.33 cm), whereas pMF under both the control treatment and 500 ppm NAA treatment showed a lateral shoot length of 2.66 cm (Fig. 7a). Additionally, pMS caused longer lateral shoots compared to pMF.

Effect of IBA treatment

In the treatment involving IBA application, the concentration of 1000 ppm in dMF exhibited the greatest lateral shoot length (10 cm), while the maximum length in dMS (8.33 cm) was recorded at the 200 ppm IBA treatment (Fig. 7b). Overall, dMS had longer lateral shoots than dMF. The IBA application at concentrations of 500 and 1000 ppm resulted in the highest lateral shoot length (7 cm) in mMS, with no significant differences between these concentrations. Additionally, in mMF, the longest lateral shoot length (3.66 cm) was associated with the application of 500 ppm IBA (Fig. 7b). For pMS and pMF, the maximum lateral shoot lengths (4 and 6.33 cm, respectively) were recorded at a concentration of 500 ppm (Fig. 7b). In general, applying IBA to *T. pubescens* increased the length of the lateral shoots compared to the application of NAA. Furthermore, these results indicated that pMS caused longer lateral shoots than pMF.

Days taken to first lateral shoots

Effect of NAA treatment

In *T. daenensis*, the mean comparison results indicated that the application of NAA increased the number of days to lateral shoot emergence in both dMS and dMF. Specifically, in dMS under the control treatment, lateral shoots emerged more rapidly than in the other species (17 d). In the NAA application test, the longest duration for lateral shoots to emerge (53.67 and 51 d) was observed at the 500 ppm concentration for dMS and dMF, respectively (Fig. 8a). The results related to *T. migricus* showed that in the NAA application test, the control treatment resulted in the lowest number of days to lateral shoots (33.16 d) in mMS, while in mMF, the shortest duration for lateral shoot emergence (21.5 d) was associated with the 1000 ppm NAA treatment. The longest time required for lateral shoot emergence (48 and 35.67 d) was recorded in the 200 ppm NAA treatment for mMS and mMF, respectively (Fig. 8a). According to the results of the present research, *T. pubescens* had a quicker emergence of lateral shoots from cuttings compared to the other species. In the NAA application treatment, the longest duration for lateral shoot emergence (33.3 d) was recorded in pMF at a concentration of 500 ppm (Fig. 8a).

Effect of IBA treatment

The application of IBA increased the number of days to lateral shoot emergence in both dMS and dMF. In the IBA application, dMS and dMF treated with 200 and 1000 ppm IBA took the longest time for lateral shoots to emerge (52 and 51.33 d, respectively) (Fig. 8b). In the IBA treatment, the shortest durations for lateral shoot emergence (45 and 33.19 d) were recorded in the 200 ppm treatment for mMS and the 1000 ppm treatment for mMF, respectively. The longest time needed for lateral shoots to emerge (58 d) was recorded in the 1000 ppm treatment for mMS, while the concentration of 500 ppm IBA in mMF took the longest time for lateral shoots to emerge (33.33 d) (Fig. 8b). According to the results of the present research, *T. pubescens* had a quicker emergence of lateral shoots from cuttings compared to the other species. In the IBA application, the longest time required for lateral shoots to emerge in pMS and pMF (33 and 33.3 d) was observed in the 500 ppm IBA treatment (Fig. 8b). The results indicated that in pMS, lateral shoots developed from cuttings more rapidly than in pMF. The data related to the effects of different concentrations of IBA and NAA on the days taken to the first lateral shoots in various *Thymus* species are presented in Table 1.

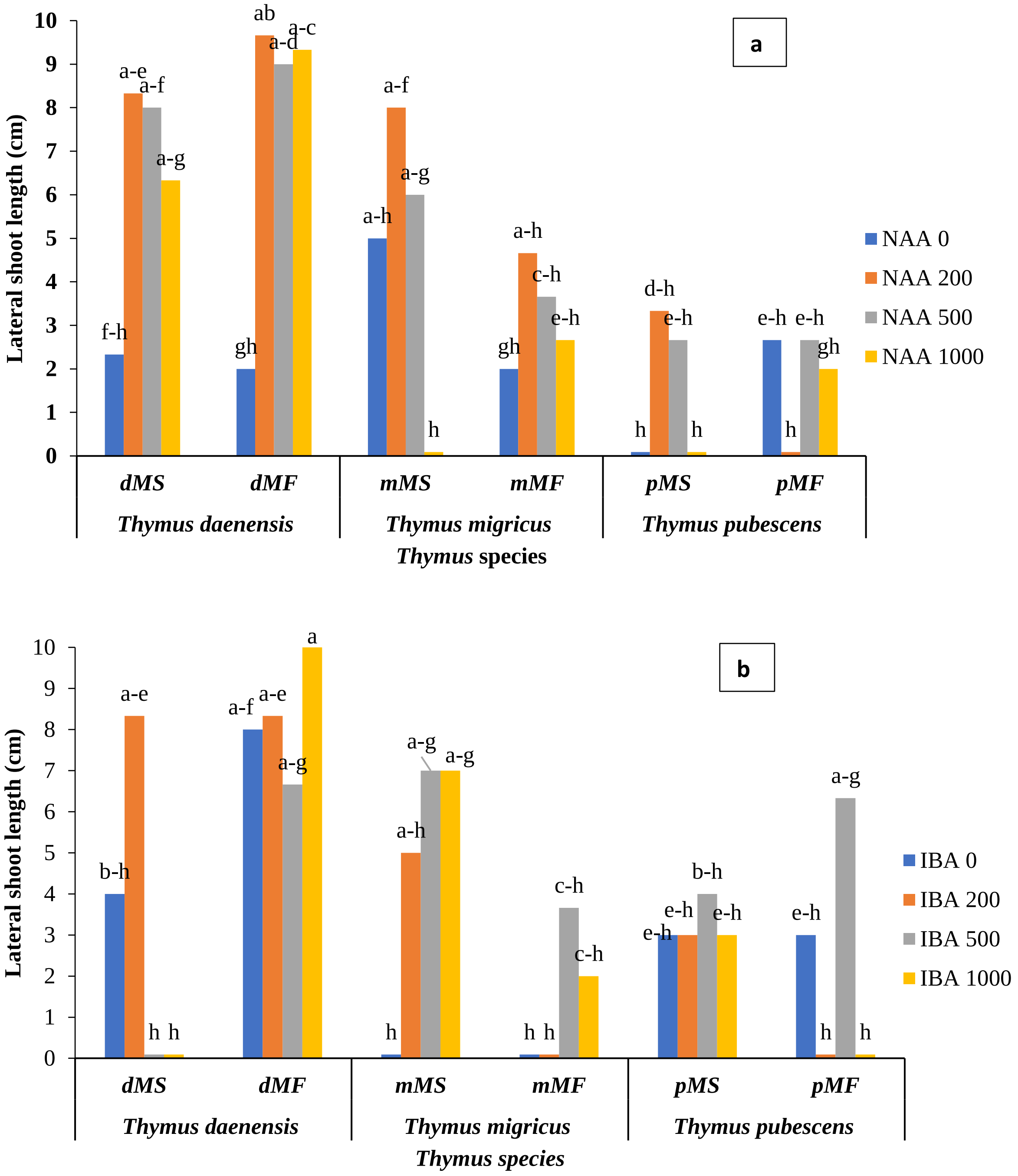

Fig. 7. Effect of different concentrations of NAA (a) and IBA (b) on the lateral shoot length of different *Thymus* species (*T. daenensis* (Male Sterile)= dMS, *T. daenensis* (Male Fertile)= dMF, *T.migricus* (Male Sterile)= mMS, *T.migricus* (Male Fertile)= mMF, *T. pubescens* (Male Sterile)= pMS, *T. pubescens* (Male Fertile)= pMF.

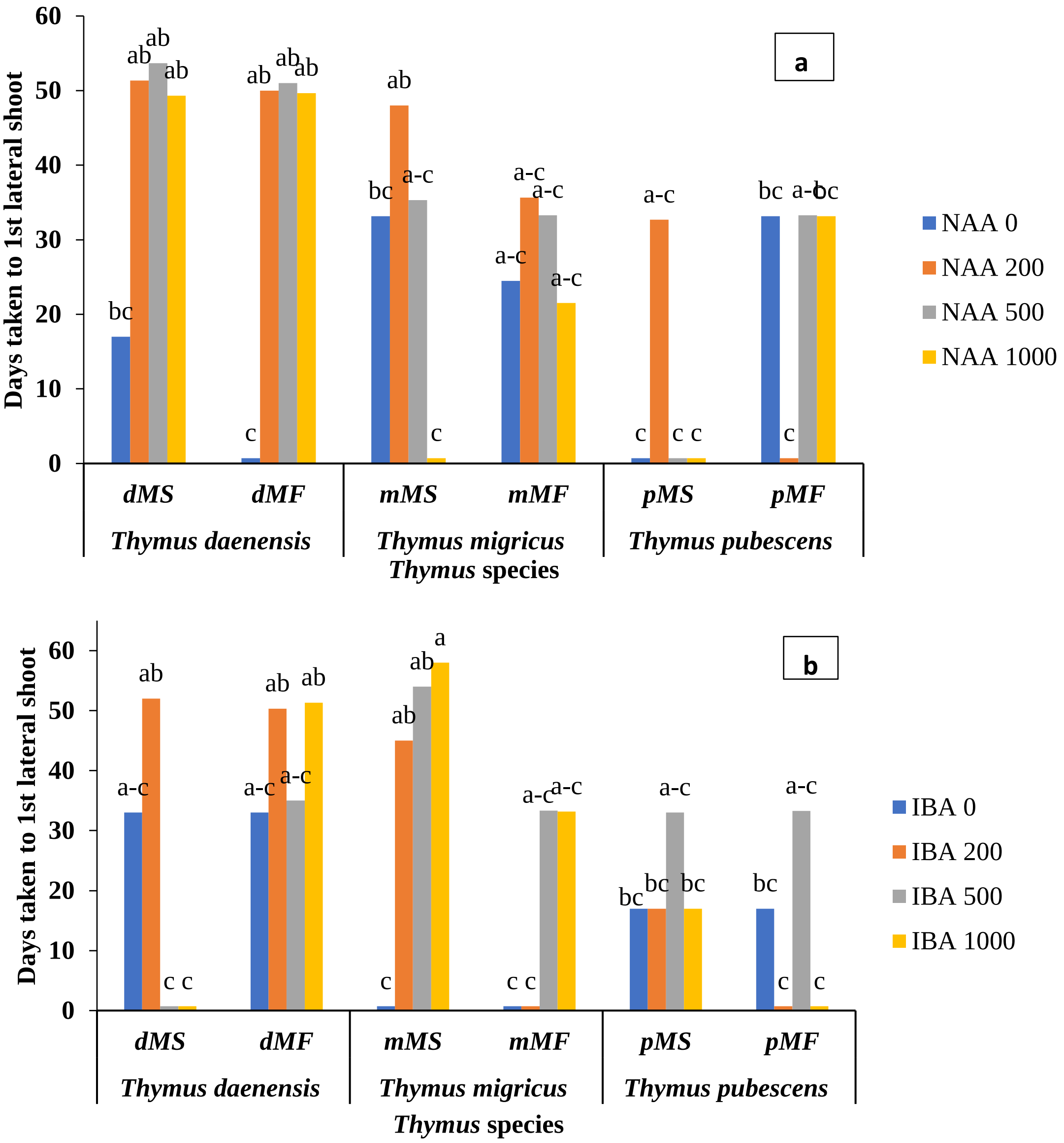

Fig. 8. Effect of different concentrations of NAA (a) and IBA (b) on days taken to first lateral shoot emergence in different *Thymus* species (*T. daenensis* (Male Sterile)= dMS, *T. daenensis* (Male Fertile)= dMF, *T.migricus* (Male Sterile)= mMS, *T.migricus* (Male Fertile)= mMF, *T. pubescens* (Male Sterile)= pMS, *T. pubescens* (Male Fertile)= pMF.

Table 1. Effect of different concentrations of IBA and NAA on days taken to first lateral shoot emergence in different *Thymus* species.

Accesions	NAA concentration (ppm)				IBA concentration (ppm)			
	0	200	500	1000	0	200	500	1000
dMS	17^{bc}	51.33^{ab}	53.67^{ab}	49.31^{ab}	33^{a-c}	52^{ab}	0.7^{c}	0.7^{c}
dMF	0.7^{c}	50^{ab}	51^{ab}	49.67^{ab}	33^{a-c}	50.33^{ab}	35^{a-c}	51.33^{ab}
mMS	33.16^{bc}	48^{ab}	35.33^{a-c}	0.7^{c}	0.7^{c}	45^{ab}	54^{ab}	58^{a}
mMF	24.5^{a-c}	35.67^{a-c}	33.3^{a-c}	21.5^{a-c}	0.7^{c}	0.7^{c}	33.33^{a-c}	33.19^{a-c}
pMS	0.7^{c}	32.67^{a-c}	0.7^{c}	0.7^{c}	17^{bc}	17^{bc}	33^{a-c}	17^{bc}
pMF	33.16^{bc}	0.7^{c}	33.3^{a-c}	33.16^{bc}	17^{bc}	0.7^{c}	33.3^{a-c}	0.7^{c}

*Different lowercase letters indicate significant differences (Duncan's multiple range test; $P \leq 0.05$).
T. daenensis (*Male Sterile)= dMS, T. daenensis* (*Male* Fertile)= dMF, *T.migricus* (*Male Sterile)= mMS, T.migricus* (*Male* Fertile)= mMF, *T. pubescens* (*Male Sterile)= pMS, T. pubescens (Male* Fertile)= pMF

Discussion

The results of the present study demonstrated that the auxin group hormones used (IBA and NAA) significantly affected the rooting of cuttings from various thyme species, each possessing different flowering systems. It is widely accepted that different auxin hormones play a critical role in initiating rooting, as they coordinate development from the cellular to the organ level and ultimately to the whole plant (Rezaei et al., 2023; Štefančič et al., 2005; Khudhur and Omer, 2015). Previous research has discussed the effects of these hormones on rooting and plant development. For example, several studies examined the effectiveness of different doses of NAA in Mountain Thyme (*Thymus kotschyanus* Boiss. & Hohen.) cuttings (Rahimi et al., 2016). Similarly, the vegetative propagation of *T. capitatus*, *T. serpyllum*, and *T. vulgaris* with the exogenous application of auxin (IBA) showed that such applications generally improved rooting in *Thymus* species (Iapichino et al., 2006). Other researchers also explored the effect of IBA on *Thymus satureioides* (Karimi et al., 2014).

In the present study, the use of rooting hormones (IBA and NAA) was found to be effective in promoting rooting across different thyme species. Although the type of hormone did not significantly impact rooting percentages among the various plants, the application of different concentrations of the hormones, compared to the control, resulted in increased rooting percentages. Our findings align with those of researchers studying *Thymus kotschyanus*, who reported that hormone type did not significantly affect rooting percentages, while the concentration of auxin did have a significant effect (Bahadori and Sharifi Ashorabadi, 2017).

The observed increase in rooting percentage following IBA treatment can be attributed to its strong role in various aspects of root development, including the regulation of root apical meristem size, root hair elongation, lateral root development, and the formation of adventitious roots (Frick and Strader, 2018). During cell-external contact, IBA induces metabolic changes in enzymes, carbohydrates, RNA, DNA, and proteins in the rooting zone, which can either inhibit or promote root growth, particularly during cell division and differentiation.

IBA and NAA are among the most widely used commercial rooting chemicals due to their non-toxic nature across a wide range of concentrations. NAA has been shown to effectively improve the survival rate of cuttings or shorten the rooting period (Meng et al., 2019). Other species of *Thymus* have also indicated that the use of auxins enhances rooting rates (Karimi et al., 2014; Khudhur and Omer, 2015; Rahimi et al., 2016). One study determined that the highest rooting rate of *Thymus revolutus* Celak reached 88.33% in cuttings treated with 500 ppm IBA (Kösa, 2021). This finding contrasts with our results, where the highest rooting percentages for nearly all species related to the treatment of 200 ppm IBA and NAA.

The results of our research demonstrated significant differences in rooting percentages among different species, with MS plants in all three species exhibiting higher rooting percentages. Previous research also observed morphological trait differences between MS and MF of *T. daenensis* and *T. pubescens* (Mohammadi et al., 2021). Additionally, in a study investigating the effects of IBA and NAA on the rooting of female and male *Laurus nobilis* L. cuttings, different rooting rates were reported between MS (0-73%) and MF (0-50%) plants. It was noted that the rooting capacity in MS was higher than that of MF (Cavusoglu and Sulusoglu, 2014), which aligned with our findings. In most species, the highest number of roots was associated with treatments of 200 and 500 ppm IBA and NAA. The number of roots in our tested plants ranged from 0.33 to 4, while a study examining the effect of NAA on *Thymus kotschyanus* Boiss. & Hohen reported a range of 7.00-9.47 roots. This discrepancy may be attributed to differences in species type or the experimental location and conditions.

Several researchers reported that various external factors, such as season, temperature, light, water, humidity, aeration, composition and pH of rooting media, as well as internal factors, including nutrients, genetic constitution, and exogenously applied rooting co-factors and hormones, influenced the rooting behavior of species (Khudhur and Omer, 2015). Nicola et al. (2003) demonstrated that the application of synthetic auxin rooting products positively affected root system development, both in terms of root number and root length. The length of the longest root ranged from 0.66 to 9.66 cm, with treatments of NAA (200 and 500 ppm) producing the longest roots in most plants. This effect may be due to auxin, at low concentrations, antagonizing inhibitory actions and stimulating root elongation (Libbert, 1955). Similarly, others observed increased root length in thyme with low auxin concentrations (Bahadori and Sharifi Ashorabadi, 2017; Rahimi et al., 2016).

Contrary to these findings, in our research, all species except dMS and pMS exhibited longer root lengths with treatments of 500 and 1000 ppm IBA compared to the control and 200 ppm IBA. This increase in root length may have been attributed to enhanced carbohydrate hydrolysis resulting from auxin treatment (Prasad, 2000). Consistent with the present study, in dMF, *Thymus satureioides* showed significant differences in average root length due to hormone treatment, with the largest root length recorded for high concentrations of auxin (500 ppm) (Karimi et al., 2014). The elongation of root length in IBA-containing treatments can be explained by the action of IBA, which breaks hydrogen bonds between cellulose microfibrils, loosening the cell wall and allowing cell elongation (Kumar et al., 2015; OuYang et al., 2015). At optimal concentrations of exogenous IBA, cambium dedifferentiation increases, hydrolytic activity accelerates, and callus formation rises, ultimately leading to improved root length (Gilani et al., 2019; Li et al., 2009).

The maximum lateral shoot length produced across different treatments could be attributed to the observation that, in most cases, the same concentrations that resulted in better root quality (length and number) also produced greater lateral shoot length. This increase may have stemmed from improved nutrient absorption by the roots and their subsequent transfer to the aerial parts of the plant. Our study's results aligned with those of other researchers (Gilani et al., 2019; Shiri et al., 2019). In support of our findings, the application of auxin for the rooting of *Thymus satureioides* cuttings demonstrated that the concentration of 500 ppm IBA yielded the highest rates and lengths of roots, as well as the greatest shoot lengths (Karimi et al., 2014).

Additionally, the differences in lateral shoot length among various species were significant. According to our results, *T. daenensis* (in both MF and MS plants) consistently exhibited greater height across all treatments than the other two species. This could be attributed to the genetic characteristics of *T. daenensis*. Our findings concurred with those of Mohammadi et al. (2020), who compared different ecotypes of *T. daenensis* with other species and concluded that *T. daenensis* had a higher height than the other studied species.

Furthermore, comparisons between MF and MS plants revealed that MF had a longer lateral shoot length than MS in *T. daenensis*, while MS plants displayed a longer lateral shoot length than MF plants in *T. pubescens* and *T. migricus*. This finding aligned with previous research comparing hermaphrodite and female plants of *T. daenensis* and *T. pubescens* (Mohammadi et al., 2021). The number of lateral shoots produced in the rooted cuttings varied among species and treatments. In tests using NAA, *T. pubescens* and *T. migricus* plants, as well as in dMS and dMF, demonstrated that the number of lateral shoots in NAA application treatments exceeded that of the control. Similarly, in tests using IBA, treatments with IBA yielded more lateral shoots than the control.

Other studies also reported the positive effects of auxin on sprouting rates (Tien et al., 2020). The accumulation of carbohydrates in cuttings promoted bud growth; therefore, the increase in the number of buds due to hormone application could be attributed to enhanced nutrient absorption resulting from a stronger root system (Shahzad et al., 2019; Wahab et al., 2001). Our findings were consistent with the established role of auxin in the sprouting of cuttings, as reported by several researchers (Kesari et al., 2009; Kontoh, 2016; Shahzad et al., 2019; Tien et al., 2020; Wahab et al., 2001).

According to the results of our research, auxin application increased the number of days taken for the first lateral shoots to emerge, which contrasted with the findings of others regarding *Phalsa* (*Grewia asiatica* L.) (Ghosh et al., 2017) and guava (*Psidium guajava* L.) cuttings (Prakash et al., 2018). Overall, the results of this research indicated that auxin application effectively improved the rooting of cuttings from different species of thyme, with the optimal concentrations for rooting being 200 and 500 ppm IBA and NAA. Additionally, a significant difference was observed in the rooting of MS and MF plants across all three species, with MS plants performing better than MF in terms of rooting.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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