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Seed quality improvement applications in black cumin seeds (*Nigella sativa* L.)

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The black cumin plant, which forms the research material, is a plant cultivated in many countries for various purposes. By eliminating the difficulties in seed germination, efficiency can be increased. Applications to improve seed quality include improving harvesting, drying and storage technologies, pre-sowing applications, seed processing and coating technologies. Among these applications, priming applications and seed coating methods occupy an important place. In previous studies on plants from the Ranunculaceae family, various pathogens of plant viruses have been identified. One of the viruses transmitted by seeds of the Ranunculaceae family is Cucumber mosaic virus (CMV). The aim was to increase germination rates and to achieve early and uniform emergence of black cumin, which has a small seed size and whose embryos also show irregular germination. For this purpose, priming, chemical application and film coating and their combinations were used to determine and recommend the best method. In addition to these studies, black cumin seeds infected with CMV were determined by serological and molecular analysis methods and a series of applications were carried out to eliminate this viral factor from the seeds. As a result of the evaluations, the seeds showed the highest germination rate (91% and 93%, respectively) when priming+polymer+KNO₃ and priming+polymer+GA₃ were applied. It was found that both polymer application and priming application increased GA₃ efficiency. Priming+polymer+GA₃ application statistically allowed the seeds to reach the mean germination time (5.87 days) in the shortest time. In contrast, the application of the polymer alone and the applications in combination with the polymer caused the seeds to reach the mean germination time longer than the control. The chemical substances and applications that successfully eliminated the cmv factor in black cumin seeds were determined. It was found that the most successful applications were hot water and ozone applications.

KEYWORDS

Nigella sativa, coating, priming, virus, ozone, seed treatment

Introduction

The development of the cultivation of medicinal plants is a situation that will be accompanied by an increase in both area and production. For this reason, it is of great importance to increase the proportion of cultivated land for medicinal plants in the existing cultivated land and to increase the amount of product obtained from a unit of land. The most important and fundamental part of agricultural production is seeds. The use of good quality seeds also leads to an increase in yield. Seed is one of the most important factors affecting yield in many species other than vegetative or tuberous species. The use of high quality seed directly increases the yield (for green parts or seeds) obtained from a unit area. Some seeds that are difficult to germinate

or have irregularities may reach the soil surface late and in low quantity under unfavourable (stress) conditions such as low and high soil temperatures and soil salinity in the environment where they are planted. This problem is very common in seeds with small seeds and small embryos. In the cultivation of medicinal and aromatic plants, various problems occur in the germination of some plant seeds. Many medicinal plants experience yield losses because germination is not fast and uniform and the soil is not used effectively (Sönmez et al., 2019). The black cumin plant, which forms the research material, is a plant that is cultivated in many countries for various purposes. In the cultivation of black cumin, there are some difficulties in the germination of the seeds. If these difficulties are removed, the efficiency can be increased.

Black cumin or black seed (*Nigella sativa* L.), which belongs to the Ranunculaceae family, is grown commercially as an annual herb and the seeds of these plants are used as an industrial raw material. The seeds are rich in fatty oil and nutrients. It has been used as a traditional medicinal healing and spice plant for many years (Arslan et al., 2011). This plant is usually cultivated in the Turkish provinces of Bursa, Afyon, Konya, Burdur and Nevşehir, and in recent years also in Southeast Anatolia (such as Gaziantep and Mardin). According to the Turkish Statistical Institute, 10,089 tonnes of black cumin were produced on 10,802 ha in 2022, 6,435 tonnes on 8,391 ha in 2021 and 3,412 tonnes on 3,377 ha in 2020 (TUIK, 2023).

Black cumin is obtained from its seeds. High quality seeds are those that are free of disease and pests, have a high germination rate and are vigorous. However, many quality problems are observed in plants that have small seeds and embryos, which also occur in black cumin, especially irregularities in germination. Therefore, one of the most important factors in achieving high yield and quality is the selection of appropriate seeds for the climatic conditions of the region. In recent years, many researchers have been working on improving seed quality to eliminate the problems caused by the seed. Duman and Zeybek (2014) applied seed quality treatments to solve these problems and achieve the desired seed quality level. Hydropriming and ZnSO₄ application on black seed under salt stress showed positive effect compared to control application (Ahmadian et al., 2015). The most important factor in black seed cultivation, as in many other species, is yield. Increasing yield is possible through the number of plants grown on a unit area and the simultaneous harvest maturity of these plants. To this end, seed improvement studies have been at the forefront of the research conducted in recent years to solve the irregular, late and low germination rates.

Seed quality improvement applications include improved harvesting, drying, processing and storage technologies, pre-sowing applications, and seed processing and coating technologies. Among these applications, osmotic conditioning (priming) and seed coating (pellet and film coating) occupy an important place (Taylor et al., 1998; Duman et al., 2011). Priming, used to achieve homogeneous and healthy germination in a short time, is defined as the controlled water uptake of the seed, which initiates the metabolic activity in the seed required for germination and does not allow rooting (Heydecker and Coolbear, 1977).

Priming is divided into four groups according to the type of application: Osmopriming, Hydropriming, Drumpriming, and Matrixpriming. Osmopriming is a method that gives better priming results for small seeds and allows controlled water uptake by the seeds in the osmotic solution, but prevents sufficient water uptake for germination (PEG, mannitol, sucrose, K, Na, Mg, etc.). Thus, enzyme activation

begins in the embryo of the seed, the embryo grows but the rootlet is prevented from leaving the testa (Sundstrom and Edwards, 1989). The application of seed coatings is one of the best methods to improve seed quality before sowing and to facilitate seed cultivation, seed colour and seed shape with the aim of applying pesticides and some plant growth regulators to the seed. There are two types of seed coating: film coating and pelleting. To improve seed quality, these two applications are combined and the use of priming and seed coating is extended.

A variety of plant pathogenic viruses can affect the growth and development of infected plants, their yield, their ornamental properties and the production of secondary metabolites used by humans. Mixed infections caused by different viruses often amplify the harmful effects of the individual viruses. Viruses can be transmitted from plant to plant by seeds, pollen, vegetative propagation and insect vectors. Plant virus diseases infecting plants from different families can seriously complicate the control of plant dispersal and cause the spread of virus infection. It is important to understand the genetic diversity, geographical distribution and biological characteristics of plant viruses to prevent their further spread (Kumari et al., 2016). The use of molecular and serological diagnostic methods can contribute to the development of effective methods in the fight against viral diseases with subsequent inactivation applications.

Seed trade, which has increased significantly between countries in recent years, is proving to be an important way to access seeds. As a result of increased cooperation between countries and the facilitation of transport, the seed industry and trade in the world have developed significantly. This diversity in seed supply has also accelerated the transmission of pathogens. Infected seeds not only lead to an increase in diseases in a region, but with the development of seed trade between countries, they also enable the spread of these diseases over long distances. As a result, it is possible for these factors to reach cultivation areas where they have not previously occurred and cause disease (Paylan and Çandar, 2020).

Transmission of viral pathogens through seeds can be high. When seeds infected with viruses are planted, the disease can easily spread through vectors, resulting in up to 100% infection in the crop. In previous studies on plants from the Ranunculaceae family, various plant virus pathogens have been identified. One of the viruses transmitted by seeds from the Ranunculaceae family is CMV, which belongs to the group of cucumber viruses, has a single-stranded RNA with an isometric particle of about 29 nm and a four-part genome. Cucumber mosaic virus can cause disease in a wide range of plants. It can infect many varieties of the Ranunculaceae family as well as black cumin plants and seeds (Guy, 2011; Nam et al., 2022).

In the last 10–15 years, studies on this topic have gained momentum worldwide, and numerous studies on seed priming and coating have been carried out, especially for commercial vegetable seeds that have economic value. However, in the case of black cumin, there is a lack of information on applications to improve seed quality. It is known that black cumin is considered as a medicinal and aromatic plant as well as an industrial crop. The studies conducted to improve the quality of black cumin seeds are also very scarce in the world.

The aim of this study was to increase the germination rates and to achieve early and uniform germination of black seed, which has a small seed size and whose embryos also have irregular germination. For this purpose, priming, chemical application and film coating and their combinations were used to determine and recommend the best method. In addition to these studies, black cumin seeds infected with

CMV were determined by serological and molecular analysis methods and a series of applications were carried out to eliminate this viral factor from the seeds.

Materials and methods

Black cumin seeds (*Nigella sativa* L.) were grown in the Aegean University Faculty of Agriculture Production Farm in the Aegean Region of Izmir, which shows the characteristics of the Mediterranean climate. These seeds have a high climate adaptability and is widely produced in the region. Black cumin seeds were obtained from Ege University Seed Technology Center. One variety and non-infected seeds were used in all treatments, except for viral disease assays and inactivation applications.

Chemical applications

KNO₃ and GA₃, which are chemicals recommended by ISTA (International Seed Testing Association) that encourage germination and eliminate dormancy, were added to the moistening media in germination and emergence tests of each type of seeds separately, and the possible effects of these chemicals were examined.

KNO₃ applications

During the sowing process, 0.2% KNO₃ application made directly in the germination and emergency stage to increase of germination and emergency rate in black cumin seeds (ISTA, 2022).

GA₃ applications

Gibberellins are plant hormones that play an important role in eliminating seed and bud dormancy, controlling and stimulating seed germination. They are found in high amounts in developing seeds. Gibberellins play a role in stimulating enzymes involved in seed germination and reducing starch to sugar in the next stage of germination (Hartmann et al., 1990). In the study, gibberellic acid was applied at a concentration of 1,000 ppm. Seeds exposed to this dose of GA₃ separately for 24 h were then subjected to a germination assay (ISTA, 2022).

Seed coating applications

Disco AG 321 (red) colored water-based polymer was used for film coating application on seeds. All seeds used in the study were covered in the "CIMBRIA" brand laboratory type CC-lab (centrikoater) seed film coating unit in Ege University Seed Technology Application and Research Center (TOTEM). During the coating process, seeds were placed in the machine from the seed inlet chamber of the machine and polymer and pure water were sent to the system with the help of a syringe. At the same time, air was supplied to the environment through the compressor connected to the machine during the process to prevent the seeds from sticking to the wall with

the coating solution. After the coating process was completed, the seeds were placed on blotting paper and left to dry at room temperature until they reached their original weight (McDonald, 2000).

Priming (pre-germination) applications

Many years of studies on osmotic seed applications have highlighted the effectiveness of the ventilated application container technique. The osmotic pressure created by the molecular weight (6000) of the PEG active substance used in pre-germination application keeps the radicle emergence under control during seed application. While PEG allow a certain amount of water to enter into the seed thanks to the pressure it creates with its heavy and high molecular weight, it does not let more water to enter into the seed as a result of the balance between the inner and outer environment of the seed and thus the radicle emergence is kept under pressure. In addition, PEG does not enter the cell wall as an effective substance and can be washed away from the seed very quickly.

In the research, osmotic seed applications, the first of the pre-sowing applications, were carried out with PEG 6000 at -1.0 MPa for 1, 2 and 3 days in a ventilated application container (Bubble-column; BK) polyethylene glycol (273 g/L) was used as the application solution. The seeds emerging from the seeds placed in the aerated application container in the permeable container were exposed to the PEG-6000 solutions separately for the times indicated above. At the end of each time, the seeds were washed in tap water for 4 min and then rinsed with distilled water. The drying process was carried out for 2 h (McDonald, 2000).

Combined applications

The best result obtained from NO₃, GA₃, priming and film coating applications were applied in combination and then germination test was started.

The seeds were coated separately with 0.2% KNO₃ solution and 0.1% GA₃ added to the polymer solution in addition to coating with polymer solution coating, the seeds were dried for 24 h. The seed coating process was also applied to seeds dried after priming. The combinations that emerged in the experiment are:

1. Control seeds
2. Polymer coating
3. Polymer coating + KNO₃
4. Polymer coating + GA₃
5. Priming
6. Priming + Polymer coating
7. Priming + Polymer coating + KNO₃
8. Priming + Polymer coating + GA₃

Germination tests

Germination tests were performed as specified in ISTA rules to determine the germination power and speed of all treated seeds and untreated control seeds (ISTA, 20). Black cumin seeds were germinated in 120×20 mm petri dishes and double-layered blotter with four replications and 100 seed per repeat. Germination test of *Nigella sativa*

L. seeds were carried out under optimum 25°C temperature conditions. The counts were made Daily during the germination tests to determine the average germination time. The seed with a rootlet of 2 mm was counted as “germinated” and removed from the petri dish.

As a results of the germination tests, the germination strength (%) value was calculated by collecting the Daily counts and taking the arithmetic average of the repeats (Larsen and Andreassen, 2004).

$$GS = \Sigma n / N \times 100$$

n: number of seeds germinating / emerging. N: total number of seeds.

In order to calculate the germination rate, the average germination time was determined in days with help of the formula stated by Pedersen et al. (1993) by using Daily counts.

$$OGT : \Sigma (dx \times nx) / \Sigma nx$$

OGT: Average germination time (G50). dx: the days of counting from the beginning of the test. nx: number of seeds germinated on the day of countin. Σnx : total number of germinating seeds.

Emerging tests

The emergence tests, carried out in parallel with the germination test, were carried out in 4 replications with 100 seeds planted in each replication. Emergency tests were carried out in plastic containers (20×12×6 cm) in sterilized and moistened soil. The seeds were sown 1–1.5 cm deep and in rows on September 1, 2021. After the sowing process, the lids of the plastic containers were closed in order to prevent moisture loss. As with the germination tests, the emergency tests were conducted at the optimum 20°C temperature. The seedlings that completed their exit from the soil environment were recorded by counting daily when the leaves were parallel to the ground, and the

trial was ended when no seedling emergence was observed for 3 days (ISTA, 2022). Emergency power (%) values were calculated by summing the daily counts as a result of the emergency tests and taking the arithmetic average of the repetitions. In the calculation of the emergence rate, the formula used as proposed in the calculation of the same germination rate (Larsen and Andreassen, 2004) was used and expressed as “Mean Germination Time” (MGT) and expressed in days.

Diagnosis of viral agent and measurement of infection levels (DAS-ELISA) – RT PCR

DAS-Elisa from serological methods and real time PCR tests from molecular methods were applied to the collected seed samples to determine viral agents in seeds. In real time PCR tests, primarily, total nucleic acids (TNA) of black cummin seeds were obtained (Figure 1). Afterwards, cdna synthesis was performed and real time PCR analyzes were performed on Roche Light Cyclor 4,680 system (Figure 2). The

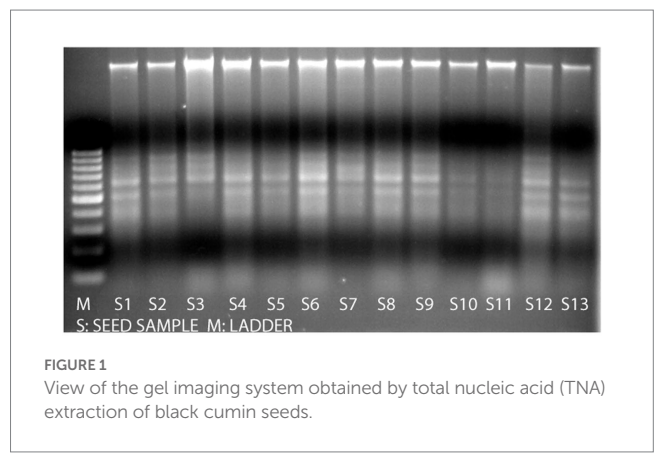


FIGURE 1 View of the gel imaging system obtained by total nucleic acid (TNA) extraction of black cummin seeds.

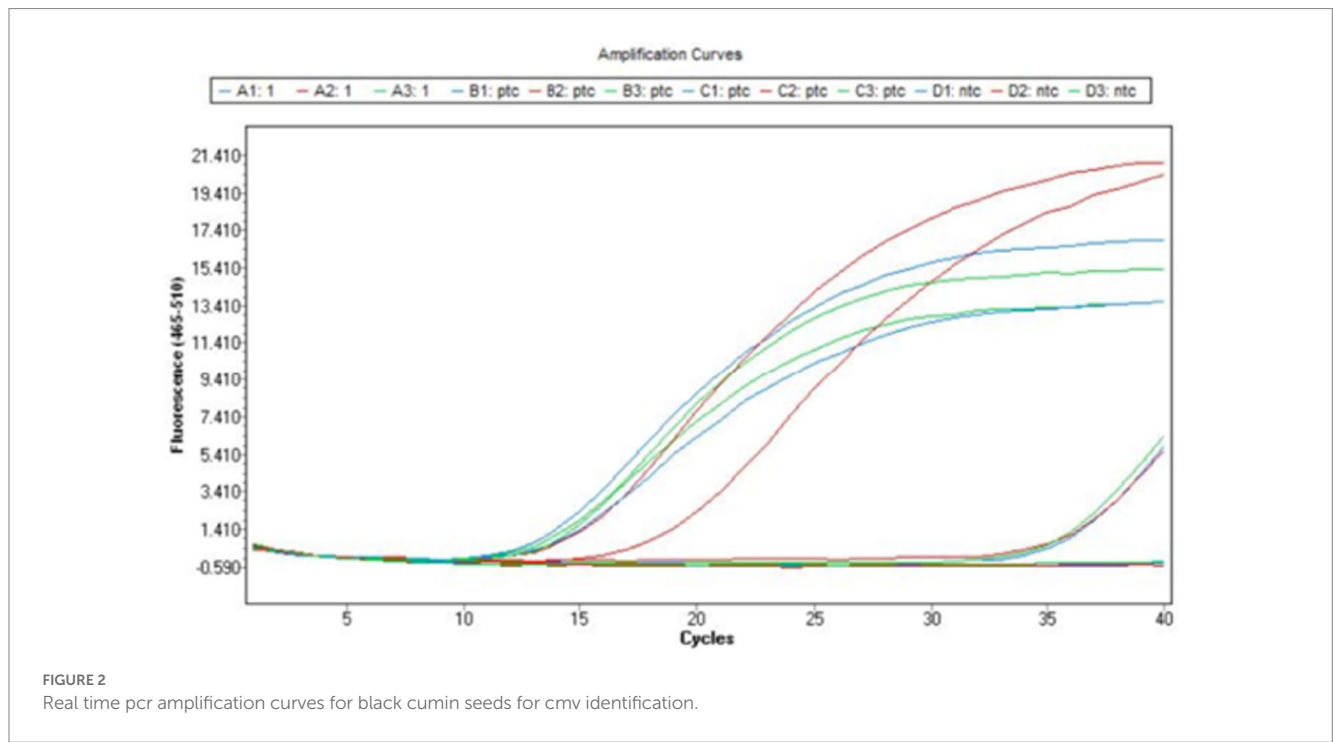


FIGURE 2 Real time pcr amplification curves for black cummin seeds for cmv identification.

change in the amount of CMV infection in seeds after inactivation applications was determined by density differences in DAS-ELISA tests with 3 replications (Nakazono-Nagaoka et al., 2005; Bhat and Siju, 2007; Nam et al., 2022; Atik and Paylan, 2023).

Viral agent inactivation treatments

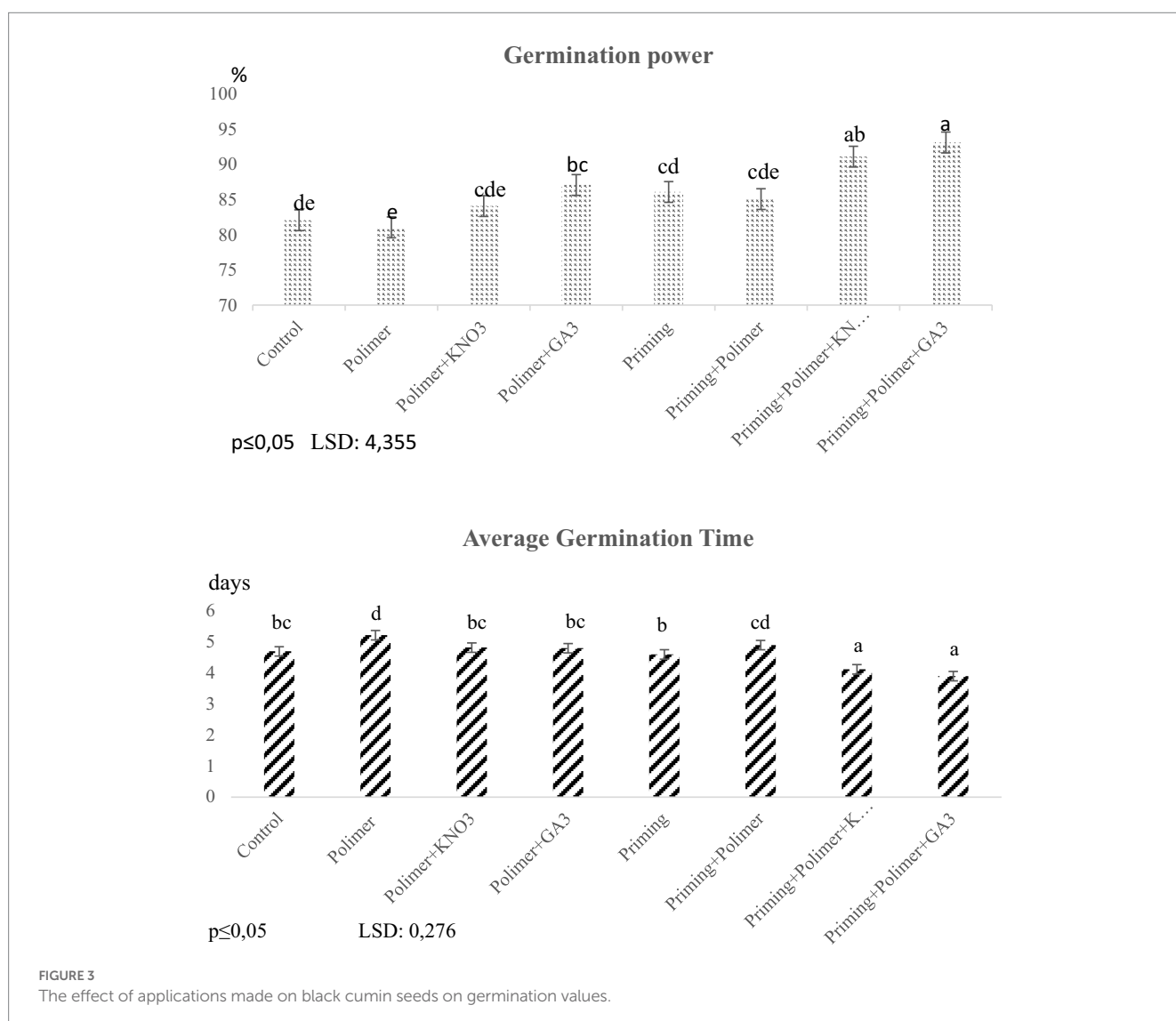
Hot Water (65°C) for 15 min, Ozone (10 min. 10 g/m³), Ozone (30 min. 5 g/m³) CH₃COOH, UV (5 min.), and H₂O₂ (4% 10 min.) applications were carried out in order to inactivate CMV on the seeds of black cumin plant determined as infected by Real Time Reverse Transcriptase PCR and DAS-ELISA Tests (Cetinkaya et al., 2022). Seed samples, which were determined to be infected with by DAS-ELISA and RT-PCR tests, were retested with DAS-ELISA method for control purposes before inactivation applications. Inactivation treatments of viral agents were carried out in 3 replications within the framework of ISTA criteria (Paylan et al., 2014; ISTA, 2022).

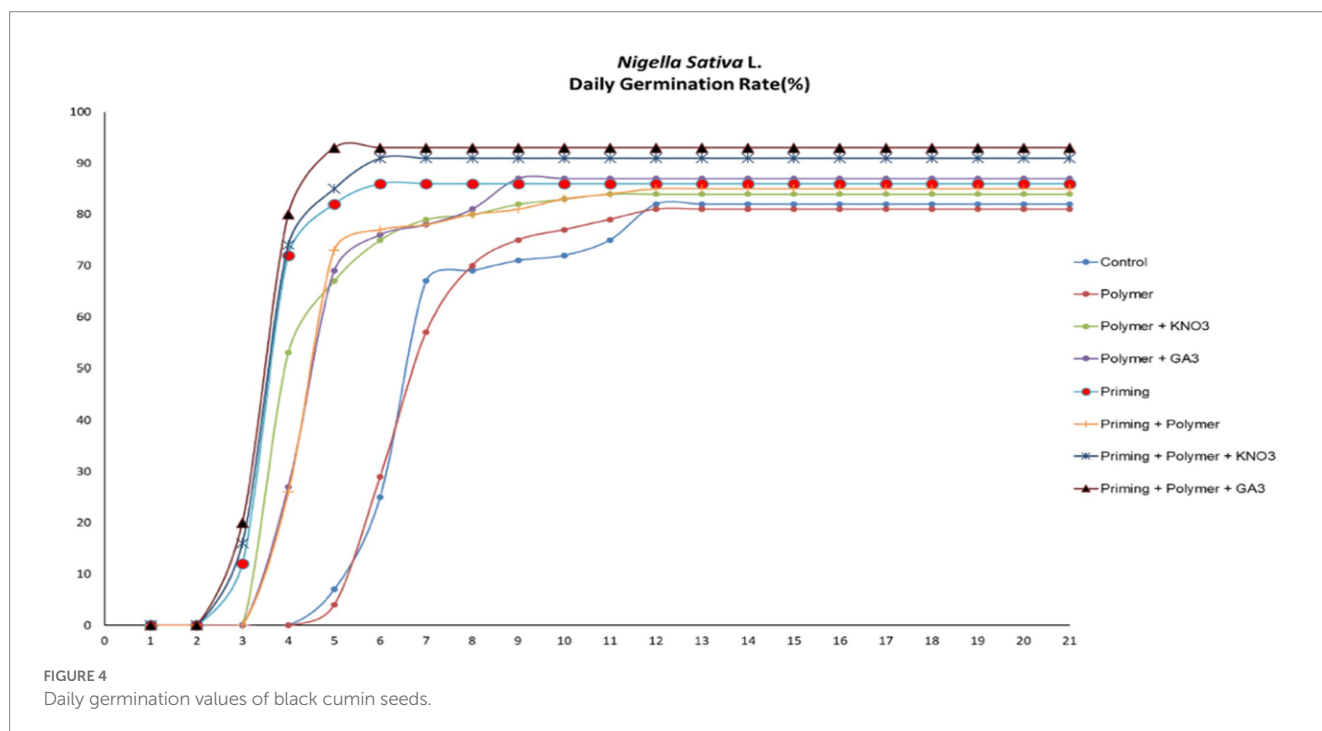
Statistical analysis

Analysis of variance (ANOVA) was computed for seed germination and growth parameters. The comparison of the treatment means was performed by LSD test ($p < 0.05$) using TotemStat (Acikgoz et al., 2004) statistical package program.

Results and discussion

The germination values obtained from the pre-sowing applications made to increase the germination performance of *Nigella sativa* L. seeds are shown in Figure 3, and the daily germination values obtained as a result of the effects on the seed germination rate are shown in Figure 4. It was determined that there was a statistically significant $p \leq 0.05$ difference between the applications in terms of the germination power (%) values obtained as a result of the applications. In the evaluation made in this respect, the seeds showed the highest rate of germination in Priming+Polymer+GA3 (93%) and





Priming+Polymer+KNO₃ (91%) applications. It is a known fact that GA₃ application has a positive effect on germination.

It has been determined that priming application accelerates germination (Asaduzzaman et al., 2021; Kayacetin, 2021) and increases the growth of seedlings and plants (Sun et al., 2010; Kayacetin et al., 2018; Sharma et al., 2018). Osmo-priming pretreatments of black cumin using -0.2 or -0.4 MPa PEG 6000 for 24 or 36 h in a medium containing -0.05 MPa PEG 6000 as post-treatment showed improved germination efficiency, with increased adaptation ability (Kayacetin, 2022). Various researchers have stated that pre-germination is not sufficient for breaking dormancy in black cumin seeds and the necessity of gibberellin application (Subaşı and Gülseven, 2010; Özcan et al., 2014; Ahmadian et al., 2015; Amir et al., 2019). It has been determined by many literature that priming, KNO₃ and GA₃, which are pre-planting pre-applications, have a positive effect on germination and mean germination time (Ajouri et al., 2004; Duman et al., 2011; Ahmadian et al., 2015). As stated in previous studies, gibberellin application to seeds increased the germination rate in this study.

The effects of the applications made to the seeds of *Nigella sativa* L. on the emergence performance are shown in Figure 5; The effects of applications on daily output values are shown in Figure 6. The findings on the emergence percentages are similar to the findings on the effects of the applications on the % germination. The highest percentage of output was obtained after Priming+Polymer+GA₃ and Priming+Polymer+KNO₃ application compared to the control (91% and 89%, respectively).

Although it varies according to the species, it is known that priming has a positive effect on the germination and emergence rates of the seeds (Harris et al., 2001; Saglam et al., 2010) and shortens the average germination/emergence time (Rowse, 1996; Kaya, 2008; Espanany et al., 2016; Duman and Gökcöl, 2018).

According to the findings obtained from inactivation studies performed on seeds infected with CMV; Hot water (65°C) application 64.7%, Ozone (10 min. 10 g/m³) application 24.3%, CH₃COOH application 17.7% and other applications with rates varying between 15.7% and 3.9% eliminated the viral agent.

While it was seen that the application of hot water (65°C) reduced the germination power to 54%, the germination values were between 75 and 90% for other applications. Average germination times are 11.66 days for hot water (65°C) application, 9.92 days for Ozone (10 min. 10 g/m³) application, 10.02 days for CH₃COOH application, 10.43 days for Ozone (30 min. 5 g/m³) application, 9.71 days for UV application, 10.19 days for H₂O₂ application and 8.88 days for control was determined as a result of the analyzes carried out (Table 1).

In previous years, it was determined that ozone in liquid and gas forms, thermotherapy and applications such as UV inactivates pathogens by 20%–70% in the studies carried out with different plants and seeds to purify them from pathogens. While some of these applications have positive effects on germination properties, some have negative effects (Paylan et al., 2014; Cetinkaya et al., 2022). In this study, also chemical substances and applications that were successful in eliminating the CMV factor in black cumin seeds were determined. It has been concluded that the most successful applications are Hot water and ozone applications. It is thought that with the combination applications of hot water and ozone applications at different doses and times, the effect of reducing the viral concentration in the seeds can be further increased and the negative effects on germination values can be reduced.

Conclusion

In this study, pre-sowing applications were made to the seeds in order to improve the germination and emergence performance of

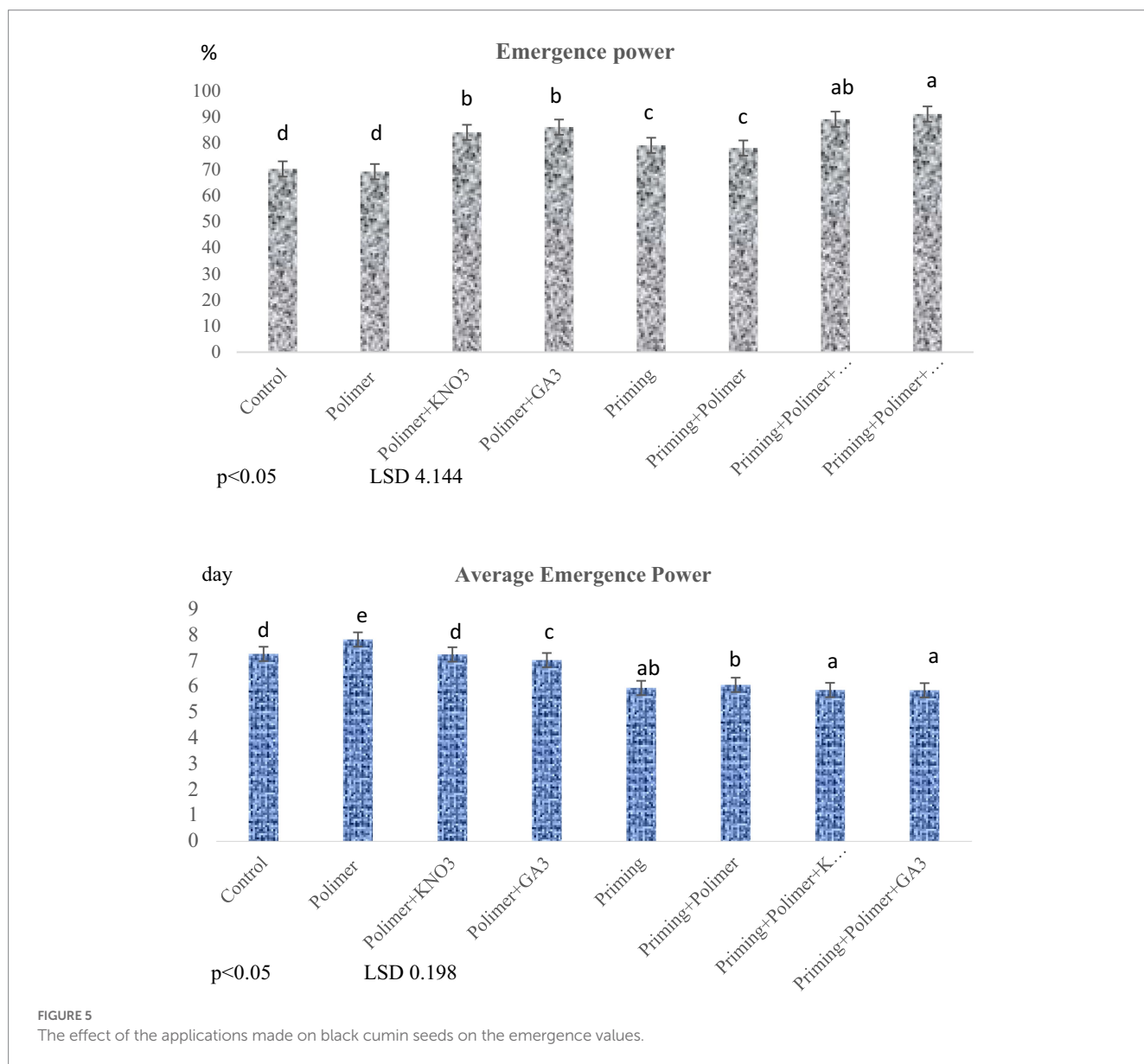


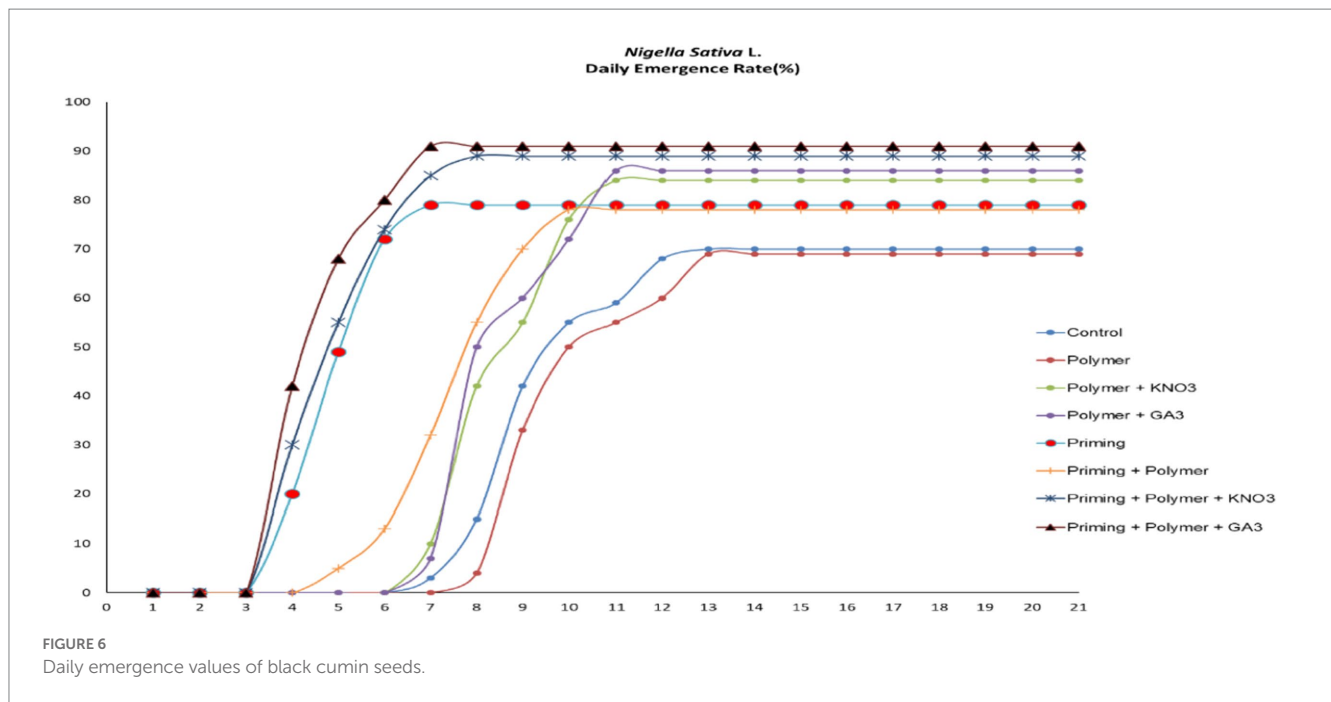
TABLE 1 Results of inactivation application to seeds infected with CMV.

Application	Elisa values		% Effect **	Germination Strength (%)		Average Germination Time (days)	
Hot Water (65 °C) 15 min.	0,397	a*	64,7	54	e	11,66	d
Ozone (10 min. 10 g/m3)	0,772	b	24,3	86	a-c	9,92	bc
CH ₃ COOH	0,837	b	17,7	79	cd	10,02	bc
Ozone (30 min. 5 g/m3)	0,864	bc	15,7	89	a-c	10,43	c
UV 5 min.	0,969	c	4,3	75	de	9,71	bc
H ₂ O ₂ % 4 10 min.	0,981	c	3,9	83	bc	10,19	bc
Control	1,329	d	-	90	a	8,88	a

* Different letters represent different statistical groups (Duncan, $p \leq 0.05$). ** Calculated using the abbott formula.

black cumin seeds and to increase their quality. For this purpose, different treatments such as Polymer coating, Polymer coating+KNO₃, Polymer coating+GA₃, Priming, Priming+Polymer coating, Priming+Polymer coating+KNO₃ and Priming+Polymer

coating+GA₃ applications were applied. By comparing the applications with the control, the effects on seed germination and emergence rate and average germination and emergence rate were examined.



As a result of the evaluations, the seeds showed the highest rate of germination (91% and 93%, respectively) in Priming+Polymer+KNO3 application and Priming+Polymer+GA3 application. Many studies have shown that GA3 application to seeds has a positive effect on germination. It was determined that both polymer application and priming application increased the GA3 efficiency. Priming+Polymer+GA3 application statistically enabled the seeds to reach the mean germination time (5.87 days) in the shortest time. On the other hand, the application of polymer alone and applications combined with the polymer caused the seeds to reach the mean germination time longer than the control.

The most important method of preventing virus diseases is to use healthy and clean production material. The identification of viruses in seed forms the basis for the measures to be taken against these diseases. Many viral diseases are transmitted through seed, and in some previous studies even certified seed was found to be infected with viral pathogens. Controlling production material such as seeds coming into the country, especially with regard to diseases, obtaining clean production material by using advanced methods such as RT-PCR in certification and quarantine programmes, ensuring that equipment in official institutions and people working with the issue are active through training studies and will use the evolving techniques. Outcomes such as accelerating the training of staff are necessary in view of the importance of the issue.

The knowledge gained from this study on seed germination and emergence has shown that the Priming+Polymer+ga3 and Priming+Polymer+kno3 applications to be used in black seed cultivation are efficient and give positive results.

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Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SE and IP: writing – methodology. AG: methodology – statistics. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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