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A NEW RAPESEED TREATMENT COMPOSITION FOR IMPROVED GERMINATION

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Abstract

Seed coating practice is used to improve plant cultivation by adding fungicides and insecticides in the form of an artificial outer layer. This technique of treatment and coating of seeds can help to improve germination and early establishment of plants. The combined application of rapeseeds treatment products with bioactive compounds and binder formulation leads to better protection of plants against pests, drought, and better ease of handling. In this context, the synthesis of a new coating composition for rapeseeds treatment was investigated. Both the raw material and the final product were analyzed using specific methods. The influence of the new coating composition on germination was studied. Experimental data indicated an increase in germination after 5 days at a temperature of 20°C for rapeseeds treated with new coating formulation which contains 18.76% hydrolyzed collagen and 3.7% thyme essential oils microencapsulates.

Key words*: coating, formulation, germination, thyme essential oil, collagen hydrolysate.*

INTRODUCTION

Seed coating is the practice of covering seeds with external materials to improve handling, protection, and, to a lesser extent, germination enhancement and plant establishment. With an annual value exceeding US$1billion dollars, this technology is mostly the preserve of the private research sector, with few links to the scientific community (Pedrini et al., 2017). Seed coating was developed as a common practice to improve plant cultivation by adding fungicides and insecticides in the form of an artificial outer layer. This practice can contribute to a certain extend to germination enhancement and early plant establishment. In addition to delivering active ingredients, artificial coatings modify the physical properties of the seeds by improving seed handling through standardization of seed weight and size. Thicker coatings are usually applied to small or morphologically uneven seeds (Chena et al., 2020).

The coating includes three techniques routinely used for seeds: film coating, encrusting and pelleting. Coating plant seeds before planting is a common practice in modern agriculture. The term "coated seed" has been applied to a seed, which was either pelleted, coated, or covered with an adhesive film. Coated seed may, in some circumstances, be produced by a dry powder process, which can have several disadvantages, such as poor adherence, non-uniform application, generation of significant amounts of dust, etc. Coating with wet powder method such as a polymer is a successful technology which can be used either singly or in a combination of other pesticides as a formulation to protect the seeds from biotic stresses like insect-pests and also reduce losses and protect from environmental concerns (Chandrika et al., 2017).

Seed coating is a mechanism involving the application of necessary materials in such a way that they affect the seed or soil at the seed–soil interface. The coating can protect the seed from many kinds of diseases and pests (Lawinska, 2019). The physical properties and thickness of the seed treatment/coating are critical factors that influence seed germination and seedling vigor. A thick hard seed coating can reduce, delay, or cause abnormal germination or may even be toxic, while a minimal, fragile seed coating can break or disintegrate before planting or not have a high enough dosage of an active ingredient to be effective (Qiu et al., 2020).

Several problems have been encountered in the development of seed treatments. For example, binder formulations that have been developed have one or more of the following disadvantages: low formulation stability, low seed flowability, high levels of dust- off of the pesticide, and other coating ingredients from the seed before planting, and poor plantability characteristics. In addition to being unpleasant for personnel who handle the treated seeds, dustiness increases potential exposure to inherent hazards of the active ingredient(s) present in the coating. Dusting also has the possible effect of reducing the efficacy of the coating due to the loss of a portion of the active ingredient(s) from the seed. Another major problem encountered in the development of binder formulations is that various ingredients that have been included in binder formulations in an effort to impart beneficial properties have an overriding negative effect on the flowability of the coated seeds (Wagner, 2013). Therefore, specialized seed coating formulations must be developed and evaluated to be utilized effectively for any given plant species and agronomic purposes (Qiu et al., 2020).

Hydrolyzed collagen (protein hydrolysate) applications were reported to enhance plant growth and yield in field tomato (Parrado et al., 2008), greenhouse tomato, papaya, maize seedlings, broccoli, and tomato seedlings. Protein hydrolysates were also reported to have ameliorating effects on abiotic stress in plants (Ertani et al., 2013). An alfalfa plant-derived biostimulant increased maize plant biomass under salinity stress, and enhanced K^+ accumulation and reduced Na^+ accumulation inroots and leaves (Ertani et al., 2013). Combined application of plant-derived protein hydrolysate and beneficial microorganisms improved lettuce root system architecture, chlorophyll synthesis, and proline accumulation and enhanced lettuce tolerance to salinity and alkalinity (Wilson et al., 2018). An alternative to primary protein resources is secondary resources such as rawhide waste from the leather industry, which is not used, although they may lead to similar performance to that of major resources and exclude contamination risks because they have already undergone severe enough chemical processing to destroy bacteria and viruses. Also, for the extraction of proteins used in agriculture, secondary collagen resources, such as by-products from the leather processing industry, represent a viable alternative (Niculescu, 2017). A collagen-based product used to coat the surface was first produced and used in the EU and Russia. Importantly, processing collagen-based materials into valuable materials for agriculture will help solve the problem of solid waste generated by the leather industry. Processing collagen-based materials into valuable materials for agriculture will help solve the problem of solid waste generated by the leather industry (Lawinska et al., 2019). Rapeseed (*Brassica napus* spp. *napus* L.) is an important oil crop ranked on third place as oil plant production, used for producing biodiesel as well as in human alimentation and in chemical industry. (Haraga et al., 2023). Rapeseed crop is a very sensitive crop to germinative seedbed preparation due to small seed dimension and a need of moist for germination. Lack of water in soil could have as effect a bad germination and low rate of cover of soil, late emergence of plants and increase susccebility of plants to dissease and pest attack (Haraga et al., 2023). To overcome some abiotic stress factors plant needs the application of product to stimulate their grow and development, increase the yield and quality (Mihailov, 2024). The biostimulants affect the rates of physiological activity to stimulate natural processes (Nickell et al, 1982; Raza et al., 2021). The main problem in the technology of winter rapessed crop comes down to overcoming abiotic and biotic stress in the early stage of development of plants wich coul leads to a failure of sown area (Mihailov, 2024).

Thyme is an aromatic plant belonging to the Lamiaceae botanical family, shows a polymorphic variation in monoterpene production, the presence of intraspecific chemotype variation being common in the genus *Thymus* (Thompson et al., 2003). Advan et all, 2006 have reveal that beneficial effect through the additive or synergistic action of several chemical. Essential oils are known to possess antimicrobial activity (Sebesan et al., 2008). Compound isolated from thyme plants (*Thymus vulgaris* L.) eliminate pathogenic microorganisms (Sebesan et al., 2008) and could be used in organic farming. This paper reports the synthesis of a new coating composition used for the rapeseeds treatment based on collagen hydrolysates, microcapsules with thyme essential oils, chelated micronutrients, and surfactants. The influence of the different type of compositions on germination of rapeseeds have been studied.

MATERIALS AND METHODS

Seeds

Rapeseeds *(Brassica napus* spp. *napus* L.) from variety Sammy with large grain (4.5-4.8 g TGW) with high oil content (40-41%) and low glucosinats content have been used in experiments.

Chemicals

Thyme essential oil (*Thymus vulgaris* L.) was purchased from Naturela, collagen hydrolysates were provided by the National Research and Development Institute for Textile and Leather - INCDTP-ICPI, the other reactive used have been procured from Scharlab SL Spain.

Synthesis of thyme essential oil microcapsules

Microencapsulation of thyme essential oil (*Thymus vulgaris* L.) was performed according to Enascuta et al., 2018, by a complex coacervation method in the first phase followed by the crosslinking phase in the presence of ultrasound (Enascuta, 2013).

Synthesis of the new coating composition for rapeseeds treatment

The new coating composition used for rape seeds treatment includes collagen hydrolysates, containing peptides with low molecular weights, having the role of stimulating the germination process; microcapsules with essential oils, with the role of biopesticide and preservation with controlled release; chelated micronutrients for plant growth and surfactants, to ensure the quality of the suspension.

The aqueous solution containing collagen hydrolysate was obtained by stirring at 500 rpm and a temperature of 30°C. In a reaction vessel containing the previously obtained hydrolyzed collagen solution (18.76%) was gradually added for 30 minutes under stirring and at room temperature 3.7% microcapsules with thyme essential oil, urea, and micronutrient chelates. For a better homogenization, the obtained bioactive composition was introduced in a blender at 3000 rpm.

Binder formulation

In a reaction vessel, the binder formulation is obtained by gradually adding under magnetic stirring polymers such as carboxymethylcellulose, hydroxymethylcellulose, starch, and polyvinyl alcohol. For a better adherence to the seeds is inserted a compound coating in the form of a powder of bentonite and dolomite.

The laboratory plant used to cover the seeds with the treatment composition consists of a mixing tank that can rotate around the axis. The speed of the mixing tank is variable, with values between 20 and 150 rotations/min.

Experiments regarding the germination effect of the rape seeds treated using the new coating composition (F1) were performed in the laboratory and the obtained values being compared with untreated rape seeds (Mt) and rape seeds treated with a coating composition with a lower concentration (F2) of collagen hydrolysates (12.3%) and microcapsules of thyme essential oils (1.2%).

After coating, the seeds were placed on filter paper dishes, 100 seeds/dish in 4 repetion, and then placed in the thermos-cabinet germinator at 20°C and 80% relative humidity in light condition. Sammy hybrid rapeseeds with TGW (Thousand Grain Weight) 4.68 g were used for testing.

Chemical characterization

The chemical composition of thyme essential oils was acquired using GC-MS/MS TRIPLE QUAD (Agilent 7890 A) with DB-WAX capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) and helium as carrier gas at 1 mL/min (Enascuta, 2018).

The morphology of the microcapsules was done using a scanning electron microscope (SEM) with a scanning electron microscope FEI QUANTA 200 (FEI Company, USA).

The new coating composition used for rape seeds treatment was analyzed in terms of total organic nitrogen (SR EN 15478:2009), ash (AOAC 920.153), density (SR ISO 758:1995), pH (SR EN 10523:2012), total sulfur (SR ISO 10084:1995) by gravimetric methods and Mn, Zn, Cu, Mg by ICP-OES method.

RESULTS AND DISCUSSIONS

Chemical composition of Thyme essential oil

The major components identified in the essential oil of Thyme essential oils were Thymol (39.29%), Isothymol (31.53%), Limonene (13.67%) and p-Cymene (8.13%), as shown in the Figure 1.

It has been observed that thyme essential oil is an important biocide; the large amount of important compounds gives the new coating composition for rapeseeds treatment both biocidal properties and preservatives properties.

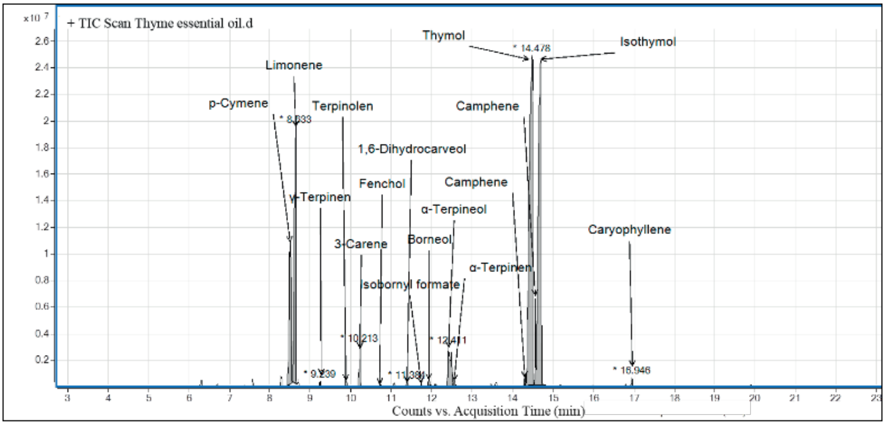

Figure 1. Chemical composition of Thyme essential oil

Chemical composition of collagen hydrolysate

The chemical composition of collagen hydrolysate was found to be the following: dry substance: 34.66%, total nitrogen: 14.8% and ash: 4.24%, protein substance: 83.18 and pH: 7.78.

Scanning electron microscope (SEM) analysis of microcapsules Thyme essential oil

Images obtained by electron microscopy looks like the rough surface of microcapsules with a few visible holes, cracks, and pores (Figure 2).

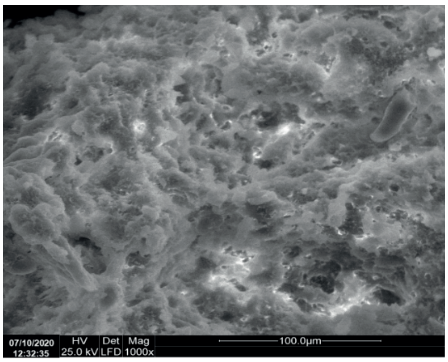

Figure 2. SEM imagines of Thyme oil microcapsules surface

Sponge-shaped structure with a large number of pores of very small size is also related to the loss of water trapped by the collagen hydrolysate network during the preparation of the sample for SEM.

Chemical characterization of the new coating composition

The values obtained from the chemical characterization of the new coating composition (F1) for the treatment of rapeseeds (Table 1) are compared with the values composition (F2) with a lower content of hydrolyzed collagen and a lower concentration of thyme oil microcapsules.

Table 1. Chemical characterization of the new coating composition

Characteristics	Values	
	F1	F2
pH	8.52	7.85
Density, g/cm^3	1.0734	1.0623
Total organic nitrogen (TKN), % (w/w)	4.76	2.76
Total sulfur, % (w/w)	0.15	0.12
Ash, % (w/w)	0.83	0.41
Mn (λ=257,610 nm), mg/kg	225	132
Cu (λ=327,393 nm), mg/kg	130	112
Zn (λ=213,857 nm), mg/kg	100	102
Mg (λ=280,271 nm), mg/kg	141	128

Influence of the new coating composition on germination

The experiences were of bifactorial type, A Factor represents the rapeseeds tested with two accessions: untreated seeds - a1, seeds coated with the new coating formulation - a2 and seeds treated with different coating formulation - a3.

B Factor represents the temperature from the growth chamber after applying the treatments of the seeds.

Because the germination values showed very large variations depending on the germination temperature, for a uniform evaluation of the impact of the treatment with the new coating composition on the germination, the determinations were performed at 5 days.

Table 2 shows the influence of treatment applied to rapeseeds with the new coating composition on germination, for each test temperature (A x B).

It was found that for the low temperatures 5°C the application of seed treatments composition determined statistically assured increases of germination, while at temperatures of 15°C and 20°C respectively although increases are recorded in all cases, germination increases are statistically ensured only in the case of the variant treated with F1.

Regarding the influence of test temperature on germination, there are significant increases in germination with increasing temperature for seeds treated with new coating composition (93.5 at 20°C).

Table 2. Influence of treatment applied to rapeseeds with the new coating composition on germination

		a_1 Mt	a_2 F1	a_3 F2
b_1 5ºC	%	66	71	68.5
	dif	Mt	5	2.5
b_2 10ºC	%	77.5	81.75	79.5
	dif	Mt	4.25	2
b_3 15ºC	%	88	91.5	89.75
	dif	Mt	3.5	1.75
b_4 20ºC	%	90.25	93.5	91.5
	dif	Mt	3.25	1.25
DL 5 %	3.125			
DL 1 %	4.238			
DL 0.1 %	6.514			

In the case of variants in which the test temperature was 5°C and 10°C, respectively, it is found that the products had the most intense impact; the tested products increase the resistance of the seeds to thermal stress.

Due to the optimal concentration of hydrolyzed collagen, the highest germination value 93.5 was observed at a temperature of 20°C for F1 while the untreated sample showed a value of germination of only 90.25. Coating of seeds with the new coating product based on thyme essential oil coarcevated and collagen hydrolysate in high concentration (F1), present a significant increase of germinative rates at all temperature levels and also an increase of germination rate comparative with coating product based on collagen with lower concentration (F2)

CONCLUSIONS

The synthesis of a new coating composition for rapeseeds treatment have been investigated. Thyme essential oil with antibacterial, antifungal and preservative properties have been analyzed using GC-MS/MS TRIPLE QUAD, the morphology of the essential oil microcapsules have been made using a scanning electron microscope (SEM) analysis and the composition of the new product have been determined using standard methods of analysis. The influence of the new coating composition on germination have been studied through laboratory experiments. Experimental data indicated a significant increase in germination after 5 days at a temperature of 20°C for rapeseeds treated with new coating formulation which contains 18.76% collagen hydrolysate and 3.7% thyme essential oils microencapsulates. For a lower qualitative germination condition (lower temperatures 5, 10 or 15°C the influence of the coating formulation (F1) have a higher influence on g process the highest influence of coating product have been reported at lowest germination temperature.

REFERENCES

Adwan, G., Abu-Shanab, B., Adwan, K., Abu-Shanab, F. (2006). Antibacterial Effects of Nutraceutical Plants Growing in Palestine on Pseudomonas aeruginosa, *Turk J. Biol., nr. 30*, 239-242.

Chandrika, K.S.V.P, Anupama, S., Prasad, R.D, Praduman, Y. (2017). Prominence of Seed Coating for Biotic and Abiotic Stresses. *Pop. Kheti*, 5(1), 44-46.

Chena, X., Krug, L., Yang, M., Berg, G., Cernava, T. (2020). Conventional seed coating reduces prevalence of proteobacterial endophytes in *Nicotiana tabacum*. *Ind. Crop. Prod.,* 155, 112784.

Enascuță, C.E., Stepan, E., Oprescu, E.E., Radu, A., Alexandrescu, E., Stoica, R., Epure, D.G, Niculescu, M.D. (2018). Microencapsulation of Essential Oils. *Revista de Chimie,* 69(7), 1612-1615.

Ertani, A., Pizzeghello, D., Altissimo, A., Nardi, S. (2013). Use of meat hydrolyzate derived from tanning residues as plant biostimulant for hydroponically grown maize. *J. Plant Nutr. Soil Sci.*, 176, 287–295.

Ertani, A., Schiavon, M., Muscolo, A., Nardi, S. (2013). Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. *Plant Soil,* 364, 145–158.

Haraga, L.C., Ion, V., Puiu, I. (2023). Effects of different sulphur rates and row spacing on oilseed rape. *Scientific Papers. Series A. Agronomy, vol. LXVI, No. 2,* 228-236.

Ławińska, K., Lasoń-Rydel, M., Gendaszewska, D., Grzesiak, E., Sieczyńska, K., Gaidau, C., Epure, D.G., Obraniak, A. (2019). Coating of Seeds with Collagen Hydrolysates from Leather Waste. *Fibres Text East Eur.*, 27:4(136), 59-64.

Mihaylov, B. (2024). Response of winter canola (*Brassica napus* L.) to treatment with growth regulators and biostimulators - A review. *Scientific Papers. Series A. Agronomy, vol. LXVII, No. 1,* 544-553.

Niculescu, M.D., Gaidău, C.C., Epure, D.G., Gîdea, M., Stepan, E. (2019). Composition based on collagen hydrolysate and method of obtaining thereof for coating leguminous plant seed. EP4388694A1, RO133376A2, 5-29.

Nickell, L. G., & Louis, N. G. (1982). *Plant growth regulators: Agricultural uses (Vol. 198).* New York: Springer-Verlag, pp. 144.

Pedrini, S., Merritt, D.J., Stevens, J., Dixon, K. (2017). Seed Coating: Science or Marketing Spin?. *Trends Plant Sci.,* 22 (2), 106-116.

Parrado, J., Bautista, J., Romero, E. J., Garciìa-Martiìnez, A. M., Friaza, V., Tejada, M. (2008). Production of a carob enzymatic extract: potential use as a biofertilizer. *Bioresour. Technol.*, 99, 2312–2318

Raza, A. (2021). Eco-physiological and biochemical responses of rapeseed (*Brassica napus* L.) to abiotic stresses: consequences and mitigation strategies. *Journal of Plant Growth Regulation, 40(4)*, 1368–1388.

Sebeşan, M., Cărăban, A. (2008). Analysis of the Essential Oils from Thyme (*Thymus vulgaris* L) and from Peppermint (*Mentha piperita* L). *Chem. Bull. "POLITEHNICA" Univ. (Timişoara) Volume 53(67), 1-2,* 212-214.

Thompson, J., Chalcha, J., Michet, A., Linhart, Y., Ehlers, B. (2003). Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *J. Chem. Ecol.,* 29(4):859-80.

Qiu, Y., Amirkhani, M., Mayton, H., Chen, Z., Taylor, A.G. (2020). Biostimulant Seed Coating Treatments to Improve Cover Crop Germination and Seedling Growth. *Agronomy*, 10(154), 1-14.

Wilson, H.T, Amirkhani, M., Taylor, A.G. (2018). Evaluation of Gelatin as a Biostimulant Seed Treatment to Improve Plant Performance. *Front. Plant Sci.,* 9, 1006.