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Seed dressing to control downy mildew of basil

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Abstract

Four experimental trials were carried out in order to evaluate the efficacy of different products (chemicals, resistance inducers and natural products) as well as heat air when applied to basil seeds naturally infested by *Peronospora belbahrii,* the causal agent of downy mildew of basil, a pathogen which is seed transmitted. Seed quality, as vigour index, was also evaluated. In general, seed treatment had a positive effect on the reduction of disease incidence and on plant biomass at the end of each the trial, with very satisfactory results when disease incidence was lower than 10% and still satisfactory results with a higher disease incidence.

Although many of the fungicides, resistance inducers and thyme oil treatments tested showed a significant disease reduction compared with the untreated control, the protection offered was only partial. Moreover, the effectiveness of the tested seed treatments varied considerably between the trials. The seeds treatment with heat air (65°C for 10 min) significantly reduced the number of infected plants per container. The interaction effect between the fungicides (at the lowest dosage tested, with the exception of acibenzolar-S-methyl, and dimethomorph), thyme oil and the heat air treatment was also significant.

In terms of biomass, most treatments not significantly improved the fresh weight in comparison with the untreated control. The highest biomass was observed in the plots where seeds were dressed with mancozeb combined with heat air. Interestingly, thiram is also effective against other pathogens affecting basil and the thermal treatment and potassium phosphite are fully compatible with the rules of organic farming. Seed dressing represents the starting point of a full integrated approach for downy mildew management.

Key words

Peronospora belbahrii; Ocimum basilicum; seed-borne pathogen; integrated control

Introduction

Peronospora belbahrii is the causal agent of downy mildew of basil, a disease observed for the first time in Uganda in the 1930s and reported as *Peronospora* sp. (Hansford, 1933) and later, in the 2000s, reported as responsible of increasingly serious losses in several countries, starting from Switzerland (Lefort *et al*., 2003), Italy (Garibaldi *et al*., 2004), Belgium (Coosemans, 2004), France (Garibaldi *et al*., 2005), and including USA (Roberts *et al*., 2009), Cuba (Martinez de la Parte *et al*., 2010), and Hungary (Nagy and Horvath, 2011). Nowadays, downy mildew has been observed wherever basil is grown as herb crop as well as for pesto sauce production. Such broad diffusion of the disease has been probably favored by the fact that the pathogen is seed transmitted (Garibaldi *et al*., 2004 a). In certain countries, such as Italy, the year 2014 has been characterized by the presence of environmental conditions very favorable to the development of the pathogen, i.e. high RH and warm temperatures (Garibaldi *et al*., 2007), with very severe epidemics and heavy field losses (Garibaldi *et al*., 2014). By considering the epidemiology of the seed transmitted *P. belbahrii* and its repercussions on control measures (Garibaldi *et al*., 2004 a; 2007; Thines *et al*., 2009), different approaches of disease management need to be evaluate. Since from the discovery of downy mildew of basil, the seriousness of the attacks and loss of production have been stimulated numerous studies in different basil production areas in the world (Gullino *et al*., 2009; McGranth *et al*., 2010; Mersha *et al*., 2012; Homa *et al*., 2014).

The management of such disease is not easy, for several reasons. First of all, being basil a minor crop, the number of chemicals registered for use is relatively low and it is not expected to increase much in the future, for economic reasons. Moreover, the use of chemicals in the field is complicated by the scalar type of harvest, which makes very difficult to comply with the intervals required between the last treatment and harvest. In fact, being the causal agent of the disease very aggressive, field sprays, even when carried out with the most effective products, provide a only partial control under severe disease pressure (Gilardi *et al*., 2013; Homa *et al*., 2014) and are not considered the wisest control methods, because of the risk of selection of strains of the pathogen resistant to the fungicides having a specific mode of action.

By considering that the pathogen is seed transmitted, one possible management strategy should be represented by seed treatment, as already shown in the case of other seed-transmitted pathogens on the same crop, i.e. *Fusarium oxysporum* f. sp. *basilici*, which is well controlled by seed dressing (Garibaldi *et al*., 1997) as well as with many other pathosystems (Gullino *et al*., 2014), including *F. oxysporum* f. sp. *lactucae* on lettuce (Lopez-Reyes *et al*., 2015).

In any case, also considering the high probability of using seed lots already infected, seed dressing should represent the first preventative strategy to be considered under an integrated disease management approach.

The present study was carried out under controlled conditions in order to evaluate the efficacy of different products (chemicals, resistance inducers and natural products) as well as heat air treatment when applied to basil seeds naturally infested by *P. belbahrii.*

Materials and methods

Greenhouse trials Four trials were carried 24-72 hours after seed treatments carried out on 27/08/2014 (trial 1), on 22/09/2014 (trial 2), on 17/09/2014 (trial 3), on 17/10/2014 (trial 4). Seeds were sown in 12 L/vol containers, using 125 seeds/plot. The substrate used was a mixture of blond peat and perlite (80:20 v/v) mix (Turco Silvestro, Albenga, Italy) previously steamed at 90°C for 30 minute. Containers were placed on benches in a greenhouse at temperatures ranging from 20 °C to 26°C with a relative humidity (RH) varying between 70 and 90%. In order to maintain HR values favourable to disease development, containers were covered with plastic. A completely randomized block design, with four replicates/treatment was used.

Seed materials Seeds of cv. Italiano classico (Furia Sementi) of basil, highly susceptible to downy mildew, belonging to the same lot which showed to be naturally contaminated by *P. belbahrii* in previous trials, were used. The seed lot used in the trials was selected because showed 7-15% of infected basil plants under completely controlled environment conditions in growth chamber at 22°C (12 h day fluorescent light regime), 15-21 days after sowing (Garibaldi *et al*., 2004). The seed sample was stored at 8°C to maintain seed a stable infection level before starting each trial.

Products tested Commercial formulations of azoxystrobin (Ortiva, 23.2% a. i., Syngenta Crop Protection), pyraclostrobin + dimethomorph (Cabrio Duo, 3.8%+6.9% a.i., BASF Crop Protection), belonging to Quinone outside inhibitors (QOIs, FRAC code 11), mandipropamid (Pergado SC, 23.4% a. i., Syngenta Crop Protection) and dimethomorph (Forum 50 WG, 50% a.i., BASF Crop Protection), belonging to carboxylic acidamides (CAAS, FRAC code 40), fluopicolid + propamocarb hydrochloride (Volare, 5.56%+55.56% a.i.,BayerCrop Sciences), belonging to the benzamides (FRAC code 43), and mefenoxam+copper (Flare GOLD R WG, a.i., 2 +14.9, Syngenta Crop Protection), belonging to phenylamides (FRAC code 4) represent the different chemicals tested.

Among multi-site fungicides, the dithiocarbamate mancozeb (Dithane DG, 75% a.i., DowAgrosciences) (FRAC code M) and the ethylene bisdithiocarbamate thiram (Tetrasol 50 WG, 47.5% a.i., Taminco Italia) (FRAC code M3) were used.

Among the resistance inducers, the mineral fertilizer based on potassium phosphite (Alexin 95PS, P_2O_5 52%, K_2O 42%, Massò), as well as acibenzolar-S-methyl (Bion 50WG, 50% a. i., Syngenta Crop Protection) of the benzo-thiadiazole group (FRAC code P) were used.

Also thyme oil (*Thymus vulgaris* 100% a. i., Soave & C, Italy) was tested.

Seed treatment Dry heat air treatment was carried out on a subsample of 4 g of basil by using the incubator Venti-Line 53,VWR brought to the temperature of 65°C. In order to perform the heat treatment, basil seeds were placed into Petri dishes (90 mm) in a single layer and maintained at 65°C for ten minutes.

Fungicides and natural compounds as well as resistant inducers were applied according to the manufacturers' instructions as wet or dry treatment by using a seed treatment equipment (Wintersteiger-Hege11, Austria).

In the wet application, used for acibenzolar-S-methyl, mandipropamid, fluopicolid+propamocarb, azoxystrobin, pyraclostrobin+dimethomorph and thiram, seeds were treated by applying a water suspension of the different products reported under table 1. Five ml of suspension/kg of seed of the different products were applied. Seeds were then dried in a sterile laminar flow chamber and used within 24-72 hours for sowing.

The dry application was performed into transparent plastic bags by shaking for 10 minutes the seeds put into transparent plastic bags with potassium phosphite, mancozeb and mefenoxam +copper. Thyme oil was applied on three absorbent filter paper (Whatman CatNo1001090) soaked in the emulsion of the product at 10%, placing into Petri dishes (90 mm diam) at the top level. Basil seeds were positioned into the same Petri dishes in a single layer and maintained at 20 ° C for 24 hours, under dark conditions.

The different products, with the exception of acibenzolar-S-methyl and fungicides based dimethomorph, were tested alone and combined with the thermal seed treatment.

Seeds were sown 24-72 hours after treatment. Samples of treated seed samples were also stored at 4 °C for further tests.

Seed quality evaluation The number of germinated seedling were counted 8 (trial 4), 10 (trials 1 and 2), 11 (trial 3) days after sowing.

The vigour test analysis was performed on treated seeds of trials 1 and 4, in order to assess the effect of different treatments under field and storage conditions. Treated and untreated seeds were checked 14-21 days after treatment, after being stored at 4°C. Vigour test trials were carried out in a germinator at 25°C by using the rolled towel paper method (ISTA, 1993). After 7 days, towel papers were removed and the length of 50 seedling, with four replicates, was measured, according to the formula: Vigour index = % of germination x seedling length (mm).

The number of irregular seedlings (because missing one or more of their essential structures such as root, the shoot or the terminal bud) germinated out of 200 was counted. The data were statistically processed as reported below.

Disease assessment Typical symptoms of downy mildew, represented by leaf chlorosis, started to be visible 17 to 26 days after sowing. Plants were checked for the presence of symptoms, starting at the cotyledon stage and affected plants per container were counted at regular intervals. Disease incidence was expressed as percentage of affected leaves at the end of the experiment (trial 1 on 26/09/2014, trials 2 and 3 on 14/10/2014; trial 4 on 14/11/2014). At the end of each trial (trial 1 on 6/10/2014, trial 2 on 14/10/2014, trial 3 on 15/10/2014; trial 4 on 19/11/2014), plants were harvested to determine the total biomass per replicate, expressed as fresh weight.

Statistical analysis All experimental data, from disease assessments (percentage of affected leaves and number of infected plants/container), from seed quality evaluations (vigour index and percentage of irregular seeds) and fresh weight (per container) were statistically processed by means of variance analysis (ANOVA) using SPSS 20.0. All data obtained from a count of health and diseased plants, were arcsine transformed to normalize their distribution. Two-Factor Analysis of variance was used to investigate the effect of fungicides, carried out at the lower dosage, thyme oil seed treatments and dry heat air treatment and their interactions. Treatments based on acibenzolar-S-methyl and dimethomorph were excluded by Two-Factor Analysis because tested alone only. Treatment means were separated by Tukey's HSD test ($P \leq 0.05$).

Results

Seed treatments (chemical treatments, with fungicides at the lower dosage, thyme oil and dry heat air treatment) had not a significant effect on seed vigour ($P=0.226$) and on the number of irregular seedlings ($P=0.157$). Also the effect of heat air treatment had not significant effect on both the seed quality parameters evaluated ($P=0.059$; $P=0.902$). The interaction effect between chemical and thyme oil treatments and dry heat air treatment was not significant too ($P=0.057$; $P=0.072$). However, significant differences were observed between the trials 1 and 4 when the number of irregular seedlings have been considered ($P<0.026$). The interaction between chemical and thyme oil treatments, dry heat air treatment, and the factor 'trial' was also significant ($P=0.004$). Among the fungicide treatments, no significant difference was observed between the dosages tested when seed quality data were considered. None of the treatments caused a significant effects on vigour index and irregular seedlings compared with the untreated seeds (Table 1).

Among trials, a significant difference was observed for seed germination ($P<0.001$). Fungicide treatments at the lowest dosage tested, and thyme oil, did not affect seed germination ($P=0.445$), as well as their combination with heat air treatment ($P=0.175$). However, the heat air treatment alone was a significant factor affecting seed germination ($P=0.021$) without interaction effect between heat air treatment and trials ($P=0.730$).

In all trials, the natural infection of seeds affected seed germination, as shown by the values of germination in the untreated control, and permitted to reach a good level of disease incidence on plants in the untreated control, as expressed by the number of affected plants/container and by the percent of affected leaves.

The good level of natural infection of the seeds, coupled with the fact that the experiments have been carried out under varying environmental conditions, permitted to evaluate the effect of seed treatments under conditions very similar to those occurring in the practice during the growing season in greenhouse (Table 1). The highest disease incidence, was observed in trial 4, with 8.4% plants affected by downy mildew, with an average of 18.4% affected leaves, at the end of the trial (Table 1). A non-significant effect was recorded among the trials when the percentage of affected leaves ($P=0.26$) and the number of infected plants ($P=0.842$) were considered. Fungicide treatments at the

lowest dosage tested, and thyme oil, significantly affected disease incidence (P<0.001). Heat air treatment was not a significant factor for the percentage of affected leaves (P=0.116), while significantly influenced the number of infected plants per container (P=0.002). The interaction effect between the fungicides (at the lowest dosage tested, with the exception of acibenzolar-S-methyl, and dimethomorph), thyme oil and heat air treatment was significant (P<0.001), also when 'trials' were considered as factor (P=0.007).

In trial 1, 7.6 plants out of 81.8 seedling showed 11.6 % of leaves affected by downy mildew in the untreated control, 38 days after sowing. Heat air treatment with thiram provided the best control. All other treatments reduced disease incidence in a statistically similar manner, without being different from the untreated control (Table 1).

In trial 2, 6.0 out of 106 seedling showed 7.8% of leaves affected by downy mildew in the untreated control (Table 1). All seed treatments carried out significantly reduced disease incidence in comparison with the untreated control. The best results were provided by the heat air treatment, followed by acybenzolar-S-methyl, mandipropamid, the heat air treatment combined with mandipropamid and with azoxystrobin (Table 1).

In trial 3, showed 4.6 plants out of 116.2 seedling showed 8.6% of leaves affected by downy mildew in the untreated control (Table 1). Seed dressing with acibenzolar-S-methyl, mandipropamid at the lowest dosage, mancozeb at the highest dosage and dimethomorph provided the best disease reduction, significantly different by the untreated control, at the end of the trial, 26 days after sowing (Table 1).

In trial 4, 8.4 plants out of 116.6 seedling showed the highest disease incidence (18.4% of affected leaves) in the untreated control (Table 1). All seed treatments carried out significantly reduced the number of infected plants compared with the untreated control. Most seed treatments significantly reduced disease incidence in comparison with the untreated control, although the protection offered was only partial. The best results, in terms of reduction of disease incidence, were provided by seed treatment with thiram at the highest dosage and potassium phosphite at the lowest dosage and by heat air treatment combined with thyme oil (Table 1).

In terms of biomass, expressed as average of the different trials, despite the high variability observed, most treatments slightly but not significantly improved the fresh weight in comparison with the untreated control. The highest plant fresh weight was observed in the plots where seeds were dressed with mancozeb combined with heat air, while the lowest values where observed with the combnation of fluopicolid+propamocarb (Figure 1).

Discussion

This work was carried out with the aim of evaluating the effect of chemical, physical and biological seed treatments and their combinations against *P. belbahrii* on basil, under greenhouse conditions. The experimental conditions adopted permitted to simulate a situation often occurring under practical conditions in geenhuse, with seeds carrying natural inoculum of the pathogen.

The present work demonstrated that the tested treatment based on fungicides, at the lowest dosage, thyme oil, heat air and their combinations did not affect seed quality parameters. Moreover, the statistical difference which has been

found between trials by considering the vigour index data, highlights a different response of basil seeds, belonging to the same seed sample, to the seed treatments carried out.

Among the many different products tested, only thiram and mancozeb are registered for use as seed treatment on basil in Italy; also natural products (such as plant extracts and mineral fertilizers) and heat air treatment are allowed. The data obtained in our work showed that the two allowed fungicides (thiram and, to a lesser extent, mancozeb) at the lowest dosage tested, the resistance inducer potassium phosphite and thyme oil, significantly reduced disease incidence. Interestingly, thiram is also effective against other pathogens affecting basil, which are seed-transmitted, such as *F. oxysporum* f. sp. *basilici* (Lopez-Reyes *et al*., in press).

Of special interest, particularly for the crops grown under the rules of organic farming, is the efficacy shown by the thermal treatment alone (heat air at 65°C for 10 min), by thyme oil and by potassium phosphite. Such treatments resulted effective and are fully compatible with the rules of organic farming. On basil, dry heat air did not affect the seed quality, as reported for cottonseed treated against *F. oxysporum* f. sp*. vasinfectum* (Bennet and Coyer, 2010). In our study, the thermal treatment carried out at 65 °C, only lasted for 10 min, in comparison with treatments at 60,70 and 80°C lasting to 14 days on cottonseed. In the present work, heat air treatment significantly influenced the number of infected plants per container. The interaction effect between the fungicides (at the lowest dosage tested, with the exception of acibenzolar-S-methyl, and dimethomorph), thyme oil and heat air treatment was also significant, without any effect of 'trial'. The protectant, curative, and antisporulant acivities of dimethomorph against several downy mildew agents is well known (Cohen *et al*., 1995). Our results indicated the ability of dimethomorph, alone or combined with pyraclostrobin, to reduce *P. belbahrii* incidence when applied as seed treatment.

Among the exploited strategies for disease management, the systemic acquired resistance inducers (SAR) are at present attracting much interest. In the present work, the effectiveness of acibenzolar-S-methyl applied as seed treatment was investigated. Previous study reported the ability of acibenzolar-S-methyl to provide reduction of downy mildew of basil when applied as leaf spray in greenhouse (Mersha *et al*., 2012; 2013; Gilardi *et al*., 2013;). Our data demonstrated that acibenzolar-S-methyl applied as seed treatment at 0.05 mg/kg of seeds significantly reduces downy mildew incidence on basil without any negative effect on seed quality.

Although many of the fungicides tested provide a significant downy mildew reduction of disease incidence in all the four trials, the protection offered was only partial. Moreover, the effectiveness of the tested seed treatments varied considerably between the trials.

With environmental conditions not too favorable to disease development, seed dressing by itself already provides significant disease control. In the presence of conditions very favorable to disease development, seed treatment must be integrated with field sprays with permitted chemicals. However, seed dressing remains the starting point of a full integrated approach for downy mildew management, also in consideration of the fact that currently varieties of commercial interest of sweet basil with resistance to downy mildew are still at the early stage (Wyenaudt *et al*., 2010; Ben-Naim *et al*., 2015).

In view of a fully IPM approach, very important in all cases and particularly for organically managed crops, among cultural practices that need to be adopted, the use of pathogen-free seed and seed dressing are very important preventative measures. The availability of non-chemical seed treatment strategies is becoming relevant and interesting for many farmers (Koch and Roberts, 2014). It is also important to avoid sprinkle (overhead) irrigation, in order to reduce relative humidity .Moreover, basil growers need to get acquainted with the first symptoms of downy mildew, in order to be able to quickly and accurately identify the pathogen, in order to adopt the correct management strategies.

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Table 1 Effect of the different seed treatments carried out against *Peronospora belbahrii* on basil (cv. Italiano) quality (expressed as Vigour index and irregular seeds, number of germinated seedlings) and on disease incidence (expressed as % of leaves affected by *Peronospora belbahrii* and number of infected plants at the end of the trials).

Treatment	Dosage a.i. /Kg of seed	Vigour index in the trial[b]				Percentage of irregular seeds in the trial[c]				Number of germinated seedlings out of 125 in trial								Percentage of affected leaves at the end of trial								Number of infected plants out of 125 in trial							
		1		4		1		4		1		2		3		4		1		2		3		4		1		2		3		4	
Untreated control	-	712	e-ha	393	e	13.0	ab	27.0	a	81.8	ab	106.0	a-d	116.2	a	116.6	ab	11.6	c	7.8	c	8.6	b	18.4	c	7.6	c	6.0	b	4.6	b	8.4	b
Dry Heat air at 65°C x10 min	-	1044	b-d	549	b-e	4.5	a-d	19.5	ab	85.6	ab	109.4	ab	120.4	a	117.0	ab	5.8	bc	1.0	a	2.6	ab	6.0	a-c	2.8	b	1.0	a	1.0	ab	1.8	a
Acibenzolar-S-methyl	0.05 mg	945	b-f	464	c-e	7.0	a-d	15.5	a-d	77.0	b	93.8	a-e	112.6	a	97.0	bc	5.6	bc	1.8	ab	1.6	a	6.3	a-c	2.8	b	1.0	ab	0.8	a	1.5	a
Mandipropamid	0.14 ml/	1107	bc	658	a-d	3.0	a-d	11.0	b-d	80.4	ab	107.2	a-c	118.0	a	105.2	a-c	2.0	ab	1.8	ab	1.6	a	8.0	a-c	1.4	ab	1.0	ab	0.8	ab	2.0	a
Mandipropamid	0.28 ml/	842	c-h	600	a-e	6.0	a-d	12.5	b-d	79.2	b	111.6	a	115.0	a	106.0	a-c	5.0	bc	3.6	a-c	2.0	ab	14.0	bc	2.8	b	1.2	ab	1.2	ab	3.0	ab
Mancozeb	0.45 g	967	b-e	631	a-e	4.0	a-d	5.5	cd	81.8	ab	101.4	a-e	113.2	a	110.6	a-c	2.3	ab	3.3	a-c	1.8	ab	5.8	a-c	1.3	ab	1.6	ab	1.0	ab	1.5	a
Mancozeb	0.9 g	873	c-h	848	a	12.5	ab	4.0	d	85.8	ab	106.4	a-d	120.2	a	113.4	a-c	2.3	ab	2.0	ab	1.4	a	7.0	a-c	1.5	ab	1.8	ab	0.8	a	1.8	a
Fluopicolide +propamocarb	0.08+0.8 ml/	1110	bc	599	a-e	5.0	a-d	3.5	d	86.2	ab	96.2	a-e	119.2	a	92.6	c	2.9	b	4.3	a-c	2.0	ab	4.5	ab	1.4	ab	1.2	ab	1.4	ab	1.5	a
Fluopicolide + propamocarb	0.16+1.6 ml/	829	c-h	596	a-e	13.5	a	10.0	b-d	89.0	ab	89.8	b-e	109.2	a	106.8	a-c	2.7	b	3.7	a-c	2.2	ab	5.3	a-c	1.4	ab	1.6	ab	1.2	ab	1.8	a
Mefenoxam + copper	0.06+0.5 g	935	b-f	591	b-e	3.5	a-d	11.0	b-d	87.6	ab	97.4	a-e	110.6	a	106.4	a-c	3.8	b	2.3	a-c	3.6	ab	4.4	ab	1.8	ab	1.2	ab	2.2	ab	1.8	a
Azoxystrobin	0.9 ml/	666	f-h	498	b-e	8.5	a-d	11.0	b-d	90.0	ab	88.8	b-e	117.0	a	119.2	a	5.7	bc	2.0	a-c	2.2	ab	5.8	a-c	3.0	b	1.2	ab	1.0	ab	1.5	a
Thiram	1.0 ml/	943	b-f	636	a-e	5.0	a-d	7.0	b-d	76.0	b	81.2	e	112.6	a	110.0	a-c	4.0	bc	3.0	a-c	2.2	ab	6.0	a-c	2.3	ab	1.4	ab	1.4	ab	1.8	a
Thiram	2.0 ml/	857	c-h	604	a-e	9.5	a-d	5.0	cd	90.8	ab	101.4	a-e	116.6	a	109.2	a-c	3.9	b	2.3	a-c	2.0	ab	1.7	a	2.2	ab	1.5	ab	1.0	ab	0.7	a
Dimetomorph	2.0 g	778	d-h	617	a-e	10.5	a-d	6.5	b-d	98.2	ab	92.0	a-e	113.2	a	106.4	a-c	7.1	bc	2.0	ab	1.4	a	5.3	ab	3.2	b	1.2	ab	0.8	a	1.7	a
Pyraclostrobin + dimetomorph	0.08+ 0.14 ml/	779	d-h	739	ab	4.0	a-d	4.0	d	87.8	ab	100.4	a-e	116.8	a	116.2	ab	3.2	b	2.2	ab	2.0	ab	4.3	ab	1.6	ab	1.2	ab	0.8	a	1.6	a
Potassium phosphite P:K 52:42	1.3+1.1 g	1168	ab	537	b-e	0.5	d	4.5	d	87.8	ab	86.2	c-e	121.6	a	110.6	a-c	2.2	b	3.3	a-c	4.6	ab	2.3	a	1.8	ab	1.5	ab	2.0	ab	1.2	a
Potassium phosphite P:K 52:42	2.6+2.2 g	1430	a	531	b-e	1.5	cd	15.5	a-d	102.6	a	89.2	b-e	119.2	a	113.4	a-c	2.7	b	2.5	a-c	2.0	ab	7.5	a-c	1.8	ab	1.4	ab	0.8	a	2.5	a
Thyme oil	10%	617	gh	506	b-e	12.0	a-c	8.5	b-d	91.6	ab	85.2	de	112.0	a	105.0	a-c	4.9	bc	5.0	bc	1.8	ab	6.8	a-c	2.6	ab	1.8	ab	1.0	a	1.8	a
65°Cx 10 min[d] + mandipropamid	0.14 ml/	987	b-e	488	b-e	1.0	d	12.0	b-d	80.8	ab	107.0	a-c	116.8	a	111.6	a-c	5.7	bc	1.5	ab	1.8	ab	4.7	ab	3.0	b	1.3	ab	1.4	ab	2.2	a
65°Cx 10 min + mancozeb	0.45 g	858	c-h	545	b-e	4.5	a-d	7.0	b-d	91.2	ab	88.8	b-e	122.2	a	114.6	ab	5.0	bc	5.7	a-c	4.4	ab	7.0	a-c	2.8	b	2.5	ab	1.8	ab	2.5	a
65°Cx 10 min + fluopicolide+ propamocarb	0.08+0.8 ml/	750	e-h	500	b-e	6.5	a-d	11.5	b-d	98.0	ab	104.2	a-d	121.2	a	118.4	ab	5.8	bc	3.5	a-c	1.6	ab	6.0	ab	2.8	b	2.3	ab	1.0	ab	1.4	a
65°Cx 10 min + mefenoxam	0.06+0.5 g	793	d-h	674	a-c	5.5	a-d	3.5	d	88.6	ab	97.8	a-e	116.2	a	115.2	ab	3.3	b	2.3	ab	1.6	ab	4.0	ab	1.4	ab	1.8	ab	0.8	a	1.7	a
65°Cx 10 min + azoxystrobin	0.9 ml/	593	h	408	de	10.0	a-d	18.5	a-c	83.2	ab	99.2	a-e	112.2	a	112.2	a-c	6.4	bc	1.8	ab	1.6	ab	6.0	a-c	2.6	ab	1.4	ab	1.0	ab	1.8	a
65°Cx 10 min + thiram	1.0 ml/	843	c-h	388	e	5.5	a-d	18.5	a-c	88.4	ab	91.8	a-e	121.6	a	117.8	ab	0.0	a	2.4	a-c	2.8	ab	4.8	ab	0.0	a	0.8	ab	1.8	ab	1.7	a
65°Cx 10 min + potassium phosphite P:K 52:42	1.3+1.1 g	878	c-h	446	c-e	2.5	b-d	10.0	b-d	92.2	ab	102.4	a-e	118.0	a	109.0	a-c	6.4	bc	3.3	a-c	2.0	ab	9.8	a-c	3.2	b	1.3	ab	1.2	ab	2.5	a
65°Cx 10 min + thyme oil	10%	889	b-g	565	b-e	5.5	a-d	3.5	d	89.6	ab	95.2	a-e	104.0	a	98.8	a-c	2.8	b	2.4	a-c	4.0	ab	3.6	ab	2.0	ab	1.2	ab	1.6	ab	1.6	a

[a] Means of the same column, followed by the same letter do not significantly differ according to Tukey's HSD test ($p \leq 0.05$) [b] Vigour Index = germination (%) x average seedlings length (mm)

[c] Irregular seeds because missing one or more of their essential structures such as root, the shoot or the terminal bud. [d] Dry Heat air.

Fig. 1 Effect of different seedtreatments against P. belbahrii onbasil (cv. Italiano) expressed asplant biomass per plot (g) at theend of the trials (average value ofthe four trials). Means of the same column, followed by the sameletter do not differ according toTukey's test (P<0.05).