Novel Liquid Mycofungicide: An Ideal Approach in Protecting Tomato from the Damping-off Disease in Nursery

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Abstract

Background: A novel liquid formulation of *Trichoderma asperellum* was prepared and its effectiveness was assessed by different methods for the management of tomato damping-off in the nursery. The experiments were conducted for two consecutive years in a randomized block design with three replications.

Results: *T. asperellum* formulation improved seed germination and management of tomato damping-off when compared with control. All the treatments performed better over control, in the first year, the antagonist's application enhanced seed germination up to 75.75 percent, however, it was 60.13 percent in control. There was 10.93 to 20.38 percent seedling mortality due to damping off which was comparatively lower than the control (26.98%). A similar trend of seed germination and disease incidence was observed in the second year. In addition to managing the disease, the antagonist certainly promoted the vegetative growth which was reflected as increased shoot and root length in comparison to control during both years. During the first year, shoot length ranged 10.90 – 12.85 cm as compared to the control (8.72 cm) and root length ranged from 3.21 to 3.65 cm which was greater than the control. Almost a similar trend in the vegetative growth parameters of seedling was observed during the second year.

Conclusion: The present investigation showed that the tested antagonist's formulation could efficiently managed the tomato damping-off as well as encouraged the vegetative growth of seedlings which ultimately ensured better and healthy seedling. And this formulation can successefully used through different methods to take care of tomato damping off.

Background

Tomato (*Solenum lycopersicum* L.) is an imperative constituent of human food basket as a good source of vitamins and minerals. It is consumed as a fresh, vegetable as well as processed products such as juice, ketchup, sauce, canned fruits, puree, paste, etc. India occupied about 789 thousand ha under tomato with a production volume of approximately 19759 thousand MT. It is also an important crop in Haryana with a 30115 ha area and production 643548 MT [1].

Having a healthy and disease-free nursery is the foremost inevitability in determining profitable crop production. Soil inhabitant fungal phytopathogens is a major challenge in the nursery raising. Species of *Pythium*, *Phytophthora*, *Rhizoctonia*, *Fusarium*, etc cause various soil-borne diseases such as damping off, root rot, wilt, etc [2, 3, 4]. Damping-off is a global problem of seedlings in the nursery, which may cause 5–80% seedling mortality of different crops [5]. Usage of synthetic fungicides had been a preferred approach since long back in managing the soil-borne fungal phytopathogens. However, issues related to human and soil health, environmental hazards, and disturbed ecosystem [6, 7, 8, 9] pressurized the agriculturists to switch over the plant protection measures towards the safer and eco-friendly approaches [10]. Biological control agents could be an ideal approach for diseases management [11, 12, 13] as they have no chance to get into the food chain and hence safe to human beings, animals, and plants [14].

Trichoderma is the most widely applied biological tool in plant protection and prospective to minimize the usage of synthetic fungicides in the agriculture sector [15]. It naturally presents in a variety of soils, decaying roots, litter, wood, bark, and other plant materials [16,17]. It alone contributes up to ninety percent of total antagonistic fungi [18] and has positive effects on the plants [19] and efficient to take care of diseases of agricultural, horticultural, ornamental crops, etc [20, 21] caused by phytopathogenic fungi [22], bacteria, and viruses [23] through various modes of actions [24]. It produces several enzymes for the degradation of the cell wall and numerous volatile metabolites [25, 26] for the control of phytopathogens. As of date, about 180 secondary metabolites (antibiotics) are characterized which inhibit or impose a lethal effect on the phytopathogens [27, 28]. Its rhizospheric application strengthens the plants to fight against aerial fungal, bacterial, and viral diseases [18] due to elicitors production and resistance induction [29]. *T. viride*, *T. harzianum*, *T. longibrachiatum*, and *T. koningii* produce volatile antibiotics [30, 31]. This antagonist creates unfavorable conditions for the phytopathogenic fungi through acidification of the environment [18].

It stimulates plant growth, persuades yield, vitamin production, boosts up nitrogen, and phosphorus availability as well as nutrients mobilization [32]. The antagonist produces zeaxanthin and gibberellins to excel in the seed germination. It also produces different acids to enhance the availability of phosphorus and other micronutrients to the plants [29]. It stimulates the local and systemic resistance in plants [33, 34, 35, 36].

It enhanced the concentration of enzymes and other important proteins for plant resistance development [37, 38]. Indigenous isolates of concerned regions performed better in that particular region [33]. Effectiveness of *T. harzianum* proven in controlling root rot (*Phytophthora capsici*) of pepper [39].

T. harzianum and *T. virens* found potential in managing the pink rot of potato and stem rot of tomato, boosted up potato tuber yield and survival of tomato seedlings [40]. Tomato, lettuce, pepper, bean, and tobacco showed resistance against *B. cinerea* owing to the application of *T. harzianum* [41]. It also induced the defense system in cucumber plants [38]. It can be used for soil application [42], seed treatment [43] mixing with farmyard manure [44], and vermicompost. It is capable of controlling the seed and soil-borne diseases of vegetables in the nursery [45]. Its 254 species are documented in the literature [46]; nevertheless, a few species have scope for control of phytopathogens [47]. Though *T. asperellum* is effective against numerous phytopathogens [48, 49] however, comparatively lesser attention has been paid for its agricultural application [50, 51].

Integration of *Trichoderma* sp in plant protection strategies could reduce the dose of synthetic fungicides and improve plant health [52] which is environment friendly as well as cost-effective [53]. Keeping into consideration the disease managing potency and plant growth promotional activity of *Trichoderma* spp, the present study was planned to test its bioefficacy against the damping-off of the tomato seedlings caused by *Fusarium* sp in the nursery.

Results

The investigation revealed that the liquid formulation of the antagonist could improve seed germination as well as reduce the damping-off in comparison with control in the nursery when it was applied in different ways, however, soil application, enriched vermicompost, and FYM found more promising.

In the first year, the seed germinated from 63.88 to 88.75 as compared to 60.13 percent in control and the seedling showed 10.93 to 20.38 percent damping off which was less than the control (26.98%) as indicated in Table 2. Likewise, in the second year, the seed was germinated from 70.83 to 89.50 percent when the antagonist was applied in various ways. The seedlings suffered from damping off disease lesser (8.38–15.33%) as compared to the control bed (20.21%) as evident from Table 3 and Fig. 2.

The antagonist positively supported the vegetative growth of the seedlings in terms of higher shoot and root length in comparison with control during both years (Fig. 2). During the first year, shoot length ranged 10.90–12.85 cm as compared to the control (8.72 cm), nevertheless, root length was observed in the range of 3.21 to 3.91 cm which was more than the control (2.70 cm). Almost a similar trend in the vegetative growth parameters of seedling was noted during the second year (Figs. 3 and 4). The maximum and minimum temperatures during 2018-19 ranged from 17.1 to 21.0 ^{0}C and 1.9 to 7.7 ^{0}C, respectively. Morning relative humidity was 90.3–99.1 and during the evening it was 48.5–65.7 (Fig. 5). During 2019 -20, the maximum and minimum temperatures ranged from 11.9 to 22.8 and 2.6 to 8.3 ^{0}C. Relative humidity during the morning was recorded 88.0 to 100 and during the evening it was 46.0–82.0 percent (Fig. 6).

Discussion

Trichoderma spp found effective in controlling the Fusarium wilt of tomato, watermelon, and muskmelon in the greenhouse and field [54]. Sowing of tomato seeds in *T harzianum* amended soil led a significant decrease in the incidence of Fusarium crown and root rot disease caused by *F. oxysporum* f. sp. *radicis-lycopersici* [55]. Researchers also assessed this antagonist for the control of this disease of vegetable crops through various application methods and noted similar findings [45].

Seed treatment, seedbed treatment, and enrichment of farmyard manure with *T. viride* and *T. harzianum* could offer a better damping-off solution of cauliflower under field conditions and improved seedling vegetative growth and health [56]. Such treatments were found effective in terms of reduced disease and improved seedling strength and health of tomato and chilli plants [57]. Tomato seed treatment with *T. asperellum* taken care of *P. aphanidermatum* [58]. Seed treatment with a consortium of *T. harzianum* significantly declined the damping-off incidence and enhanced the shoot length, chlorophyll content, and yield [59]. Inoculation of tomato seed and nursery soil with *T. harzianum* spore suspension laid increment in the shoot as well as root length, diameter, and weight when compared to the control. There was a noticeable increase in leaf number, leaf area, chlorophyll content [60].

Application of *T. asperellum* in field conditions, managed phytopathogenic *Fusarium* sp, and significantly increased the vegetative growth in respect of root length, shoot length, plant weight as well as chlorophyll content. It also improved total phenol, peroxidase, polyphenoloxidase, and phenylalanine ammonium lyase activity which developed plants resistance against the pathogen [61]. Pre-sowing application of *T. viride* (WP) in nursery beds of tomato showed a reduction in disease incidence. Nevertheless, growth parameters showed a positive impact [62]. Soil application of *T. viride* efficiently controlled the pre- and post-emergence damping-off of tomato (*P. aphanidermatum*) and improved vegetative growth of the plant [63]. *T. harzianum* and *T. virens* treated tomato seed revealed up to cent percent seed germination and increased root and shoot length [64].

Trichoderma amended compost could effectively suppress root disease causal fungal phytopathogens viz., *F. oxysporum*, *P. debaryanum*, *P. aphanidermatum*, *R. solani* in tomato and persuaded seed germination, seedling height, and overall biomass of seedling [65]. *T. harzianum* enriched organic matter could reduce the tomato damping-off intensity and increase seedling growth [66]. Concurrent application of *T. harzianum* as seed, seedbed, and soil treatment performed better than its soil application in terms of disease management and better growth parameters of eggplant and tomato. Vermicompost enriched with antagonist increased seed germination, promoted seedling vegetative growth, and minimized damping-off incidence [67]. The disease control and plant growth-promoting property of *T. asperellum* were established against *R. solani* in cucumber plant [68]. Inoculation of *Trichoderma* in tomato nursery and field could improve plant health in terms of increased the shoot weight, fruit size, yield [69]. Furrow application of *T. harzianum* in the field conditions could reduce black scurf incidence in organically grown potatoes [70]. A few *Trichoderma* strains showed good control of *P. ultimum* in pea and plant growth promotional property in terms of increased shoot weight, root weight, root length number of lateral roots [71].

Methods

Experimental location

The nursery trials were carried out at the research farm at google map position: 29.153843 NS Latitude and 75.694118 EW Longitude, Department of vegetable science, Chaudhary Charan Singh Haryana Agricultural University, Hisar for two consecutive years during 2018-19 and 2019-20.

Preparation of mycofungicide formulation

The liquid formulation (10% Aqueous Suspension) of *T. asperellum* (KBN-29, accession number ITCC-7764) was prepared following a slightly modified method [72, 58]. The pure culture of the antagonist was inoculated into potato dextrose agar (HiMedia India Pvt. Ltd.) plates, followed by proper sealing with parafilm under aseptic conditions and incubated at 26 ± 2^0C for 5 days in a BOD incubator. Ten milliliters double-distilled sterilized water was added to each plate and fungal biomass (conidia and mycelia) was scrapped with the help of scrapper. The harvested biomass was further diluted with water and a conidial concentration of approximately $2x10^9$ per ml was determined through serial dilution plate inoculation technique (Fig. 1). The prepared formulation was kept in the refrigerator and used within a month.

Source of plants

The locally cultivated tomato var. Selection-7, released by the Department of Vegetable Science, Chaudhary Charan Singh Haryana Agricultural University was used for experimentation. Seed of this variety was obtained from the Vegetable seed store of this Department.

Assessment of different application methods against damping off

The developed formulation was assessed as a seed treatment, soil application, drenching, enriched FYM, and vermicompost. The antagonist treated seed was dried under shade. In soil application, the formulation was sprayed uniformly in to plot before 4-5 hr. It was mixed with FYM and vermicompost before 15 days of its field application and kept covered under shade. Carbendazim was taken as a standard check in the experiment. There were seven treatments in three replications (Table 1). Raised seedbeds of 90 cm x 45 cm dimensions were prepared in mid-December, 2018, and 2019 (Fig. 2). Two hundred tomato seeds per treatment were sown manually. After 15 days damping-off incidence was recorded. Then drenching of antagonist and carbendazim was done in respective treatments and again after two weeks, disease observation was recorded in comparison with control. After about 45 days of nursery raising, plant growth in terms of shoot and root length (cm) was measured from ten randomly sampled seedlings per treatment.

Data analysis

The experimental data were statistically analyzed through the online package ‘OPSTAT’ of the institute.

Weather conditions during the study period

Weather data of temperature, relative humidity, wind speed, sunshine hours, and rainfall were collected from the Department of Agrometeorology, College of Agriculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Conclusion

The present investigation showed that the tested antagonist’s formulation could efficiently managed the tomato damping-off as well as encouraged the vegetative growth of seedlings which ultimately ensured better and healthy seedling. And this formulation can successefully used through different methods to take care of tomato damping off.

Abbreviations

TSA-*Trichoderma* soil application, TD- *Trichoderma* drenching, TST- *Trichoderma* seed treatment, TE-FYM-*Trichoderma* enriched FYM, CarbD-Carbendazim drenching, TEV- *Trichoderma* enriched vermicompost

Declarations

Ethics approval and consent to participate

The study was conducted with tomato plant species those are abundant in the ecosystem hence do not require ethical approval.

Consent for publication

The authors agree to publish this paper. The data has not been published partially or completely in any other journal.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to privacy issues but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper. It is declared that the authors have no competing interests.

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Authors' contributions

Kishor Chand Kumhar generated the idea of the study, prepared mycofungicide formulation, decided treatments, recorded disease incidence, analyzed, and presented data in tables and graphs. Kuldeep Kumar arranged seed, raised nursery, and recorded data on growth parameters. Indu Arora has taken care of the nursery during the study period. Arun Kumar Bhatia and Vinod Kumar Batra timely monitored and suggested the required follow up action.

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Tables

Table 1

Dose and methods of application for the management of tomato damping-off

Treatment	Description	Dose
T1	Soil application of *T. asperellum*	1 ml /l
T2	Soil drenching of *T. asperellum*	1 ml /l
T3	Seed treatment *T. asperellum*	1 ml /kg
T4	FYM *T. asperellum*	250 ml / kg
T5	Vermicompost *T. asperellum*	250 ml / kg
T6	Carbendazim drenching	2.5 g /l
T7	Untreated control	-

Table 2

Evaluation of *Trichoderma asperellum* for management of seedling disease of tomato under nursery (2018-19)

Treatment	Germination (%)	Damping-off incidence (%)
T1-Soil application of *T. asperellum*	75.75 (60.51 ± 1.00)	11.37 (19.64 ± 0.92)
T2-Soil drenching of *T. asperellum*	63.88 (53.10 ± 2.08)	20.38 (26.78 ± 1.16)
T3-Seed treatment *T. asperellum*	65.50 (54.03 ± 1.23)	19.19 (25.86 ± 1.59)
T4-FYM *T. asperellum*	72.00 (58.06 ± 1.03)	13.36 (21.43 ± 0.42)
T5-Vermicompost *T. asperellum*	88.75 (70.86 ± 2.69)	10.93 (19.21 ± 1.14)
T6-Carbendazim drenching	73.00 (58.79 ± 2.10)	12.08 (20.03 ± 2.22)
T7-Untreated control	60.13 (50.85 ± 1.54)	26.98 (31.22 ± 1.49)
CD ($p < 0.05$)	5.27	3.55
***Average of 3 replications, figures in parenthesis is angular transformed values ± SE**		

Table 3

Bioefficacy of *Trichoderma* liquid formulation against tomato damping-off in the nursery at the vegetable farm (2019-20)

Treatment	Germination (%)	Damping-off incidence (%)
T1-Soil application of *T. asperellum*	83.17 (65.86 ± 1.89)	8.38 (16.72 ± 1.50)
T2-Soil drenching of *T. asperellum*	70.83 (57.34 ± 1.83)	15.33 (23.03 ± 0.74)
T3-Seed treatment *T. asperellum*	71.00 (57.48 ± 2.34)	14.43 (22.28 ± 1.08)
T4-FYM *T. asperellum*	75.67 (60.45 ± 1.16)	11.68 (19.95 ± 0.53)
T5-Vermicompost *T. asperellum*	89.50 (71.19 ± 1.64)	7.82 (16.09 ± 1.71)
T6-Carbendazim drenching	81.00 (64.21 ± 1.65)	8.54 (16.90 ± 1.26)
T7-Untreated control	66.83 (54.83 ± 1.24)	20.21 (26.69 ± 0.58)
C.D. ($p < 0.05$)	3.52	3.78
***Average of 3 replications, figures in parenthesis is angular transformed values ± SE**		

Figures

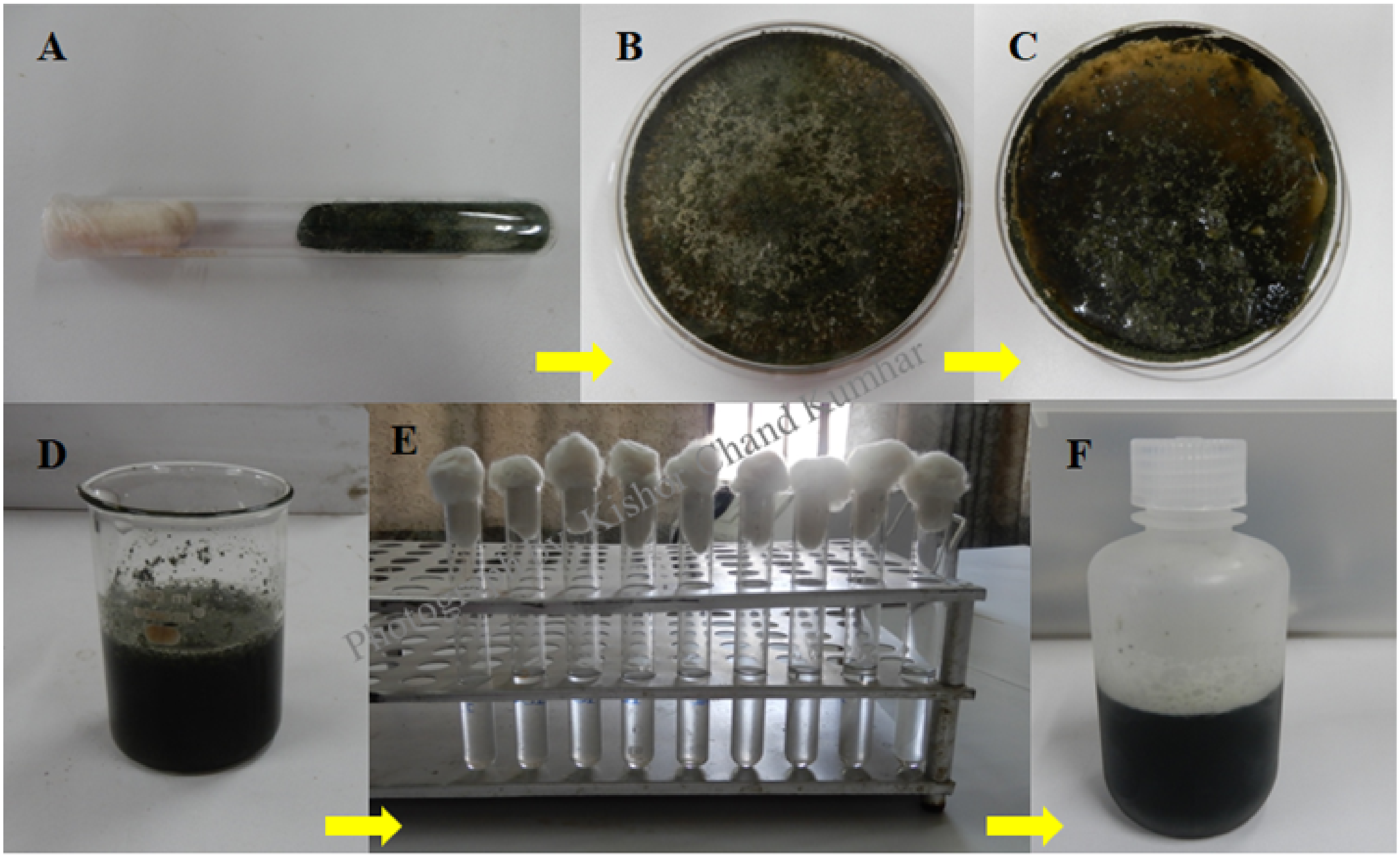

Figure 1

Steps of product formulation: A-Pure culture slant, B- Culturing on PDA plate, C- Biomass scrapping, D- Biomass collection, E-Serial dilution and F- Liquid formulation

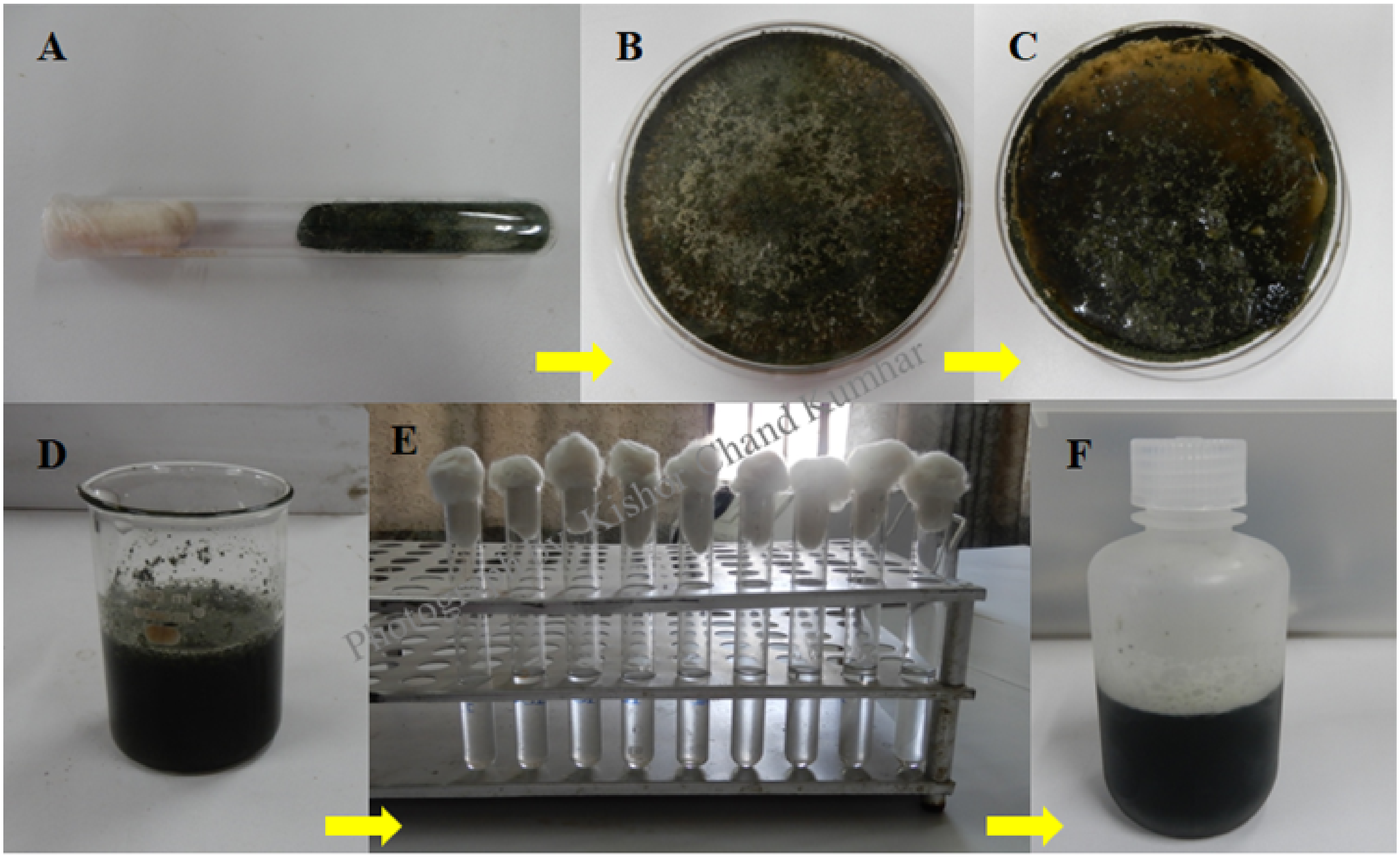

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Figure 2

Performance of T. asperellum in tomato nursery: A- post emergence damping off, B-vegetative growth (arrow indicating control bed)

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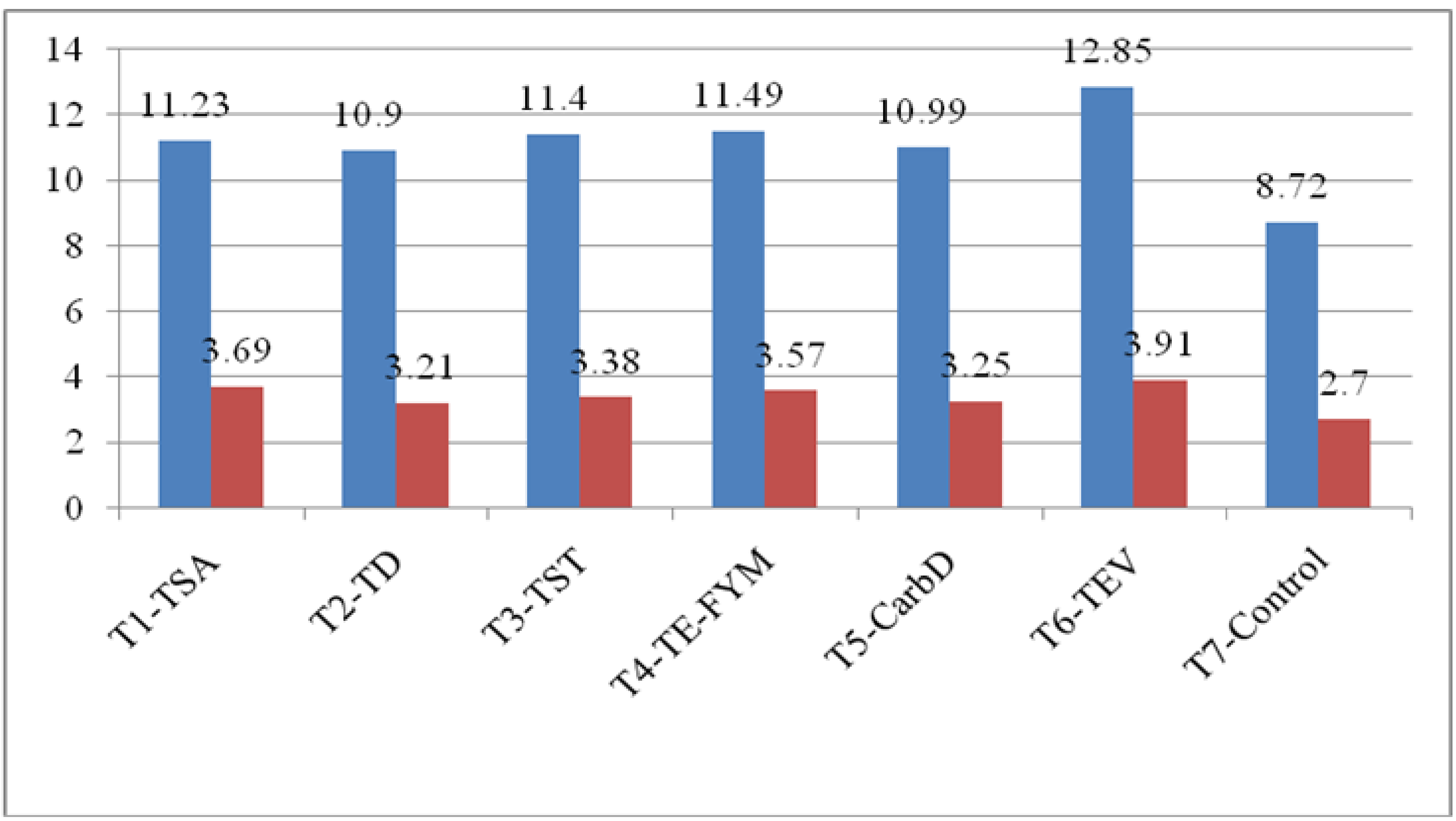
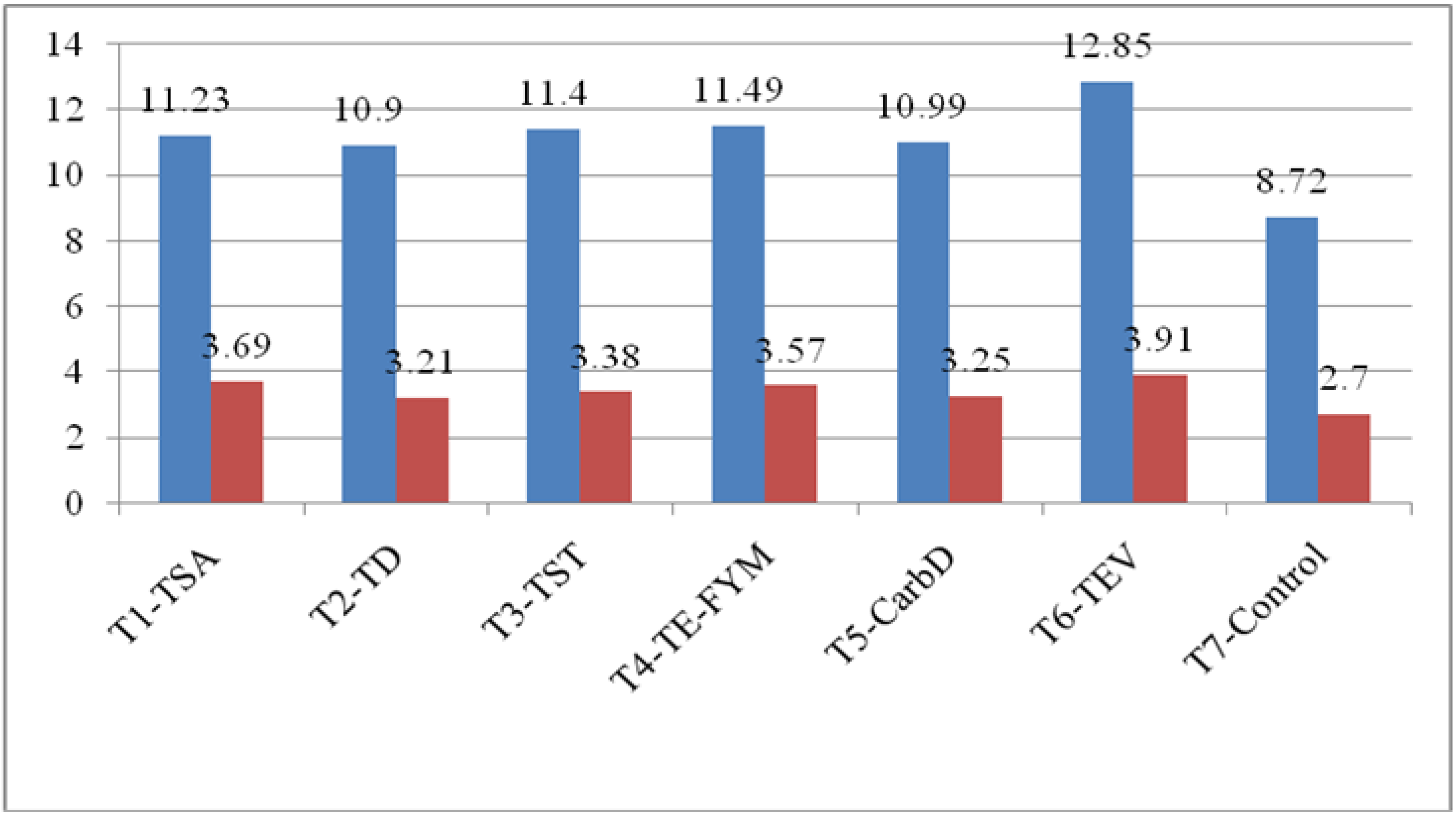


Figure 3

Effect of Trichoderma formulation on shoot and root growth of tomato in nursery (2018-19) Legends: Shoot length (cm) Root length (cm)



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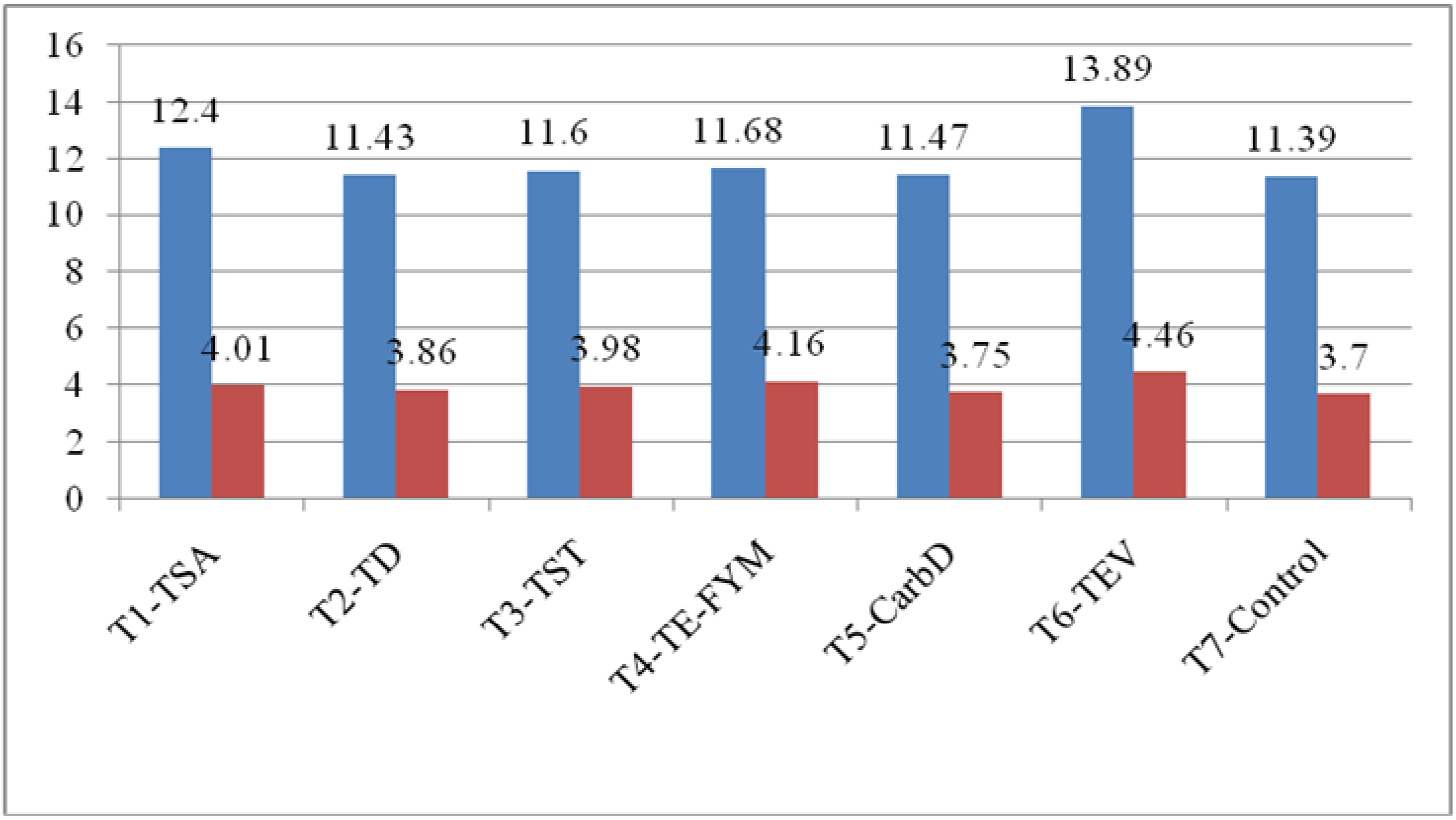
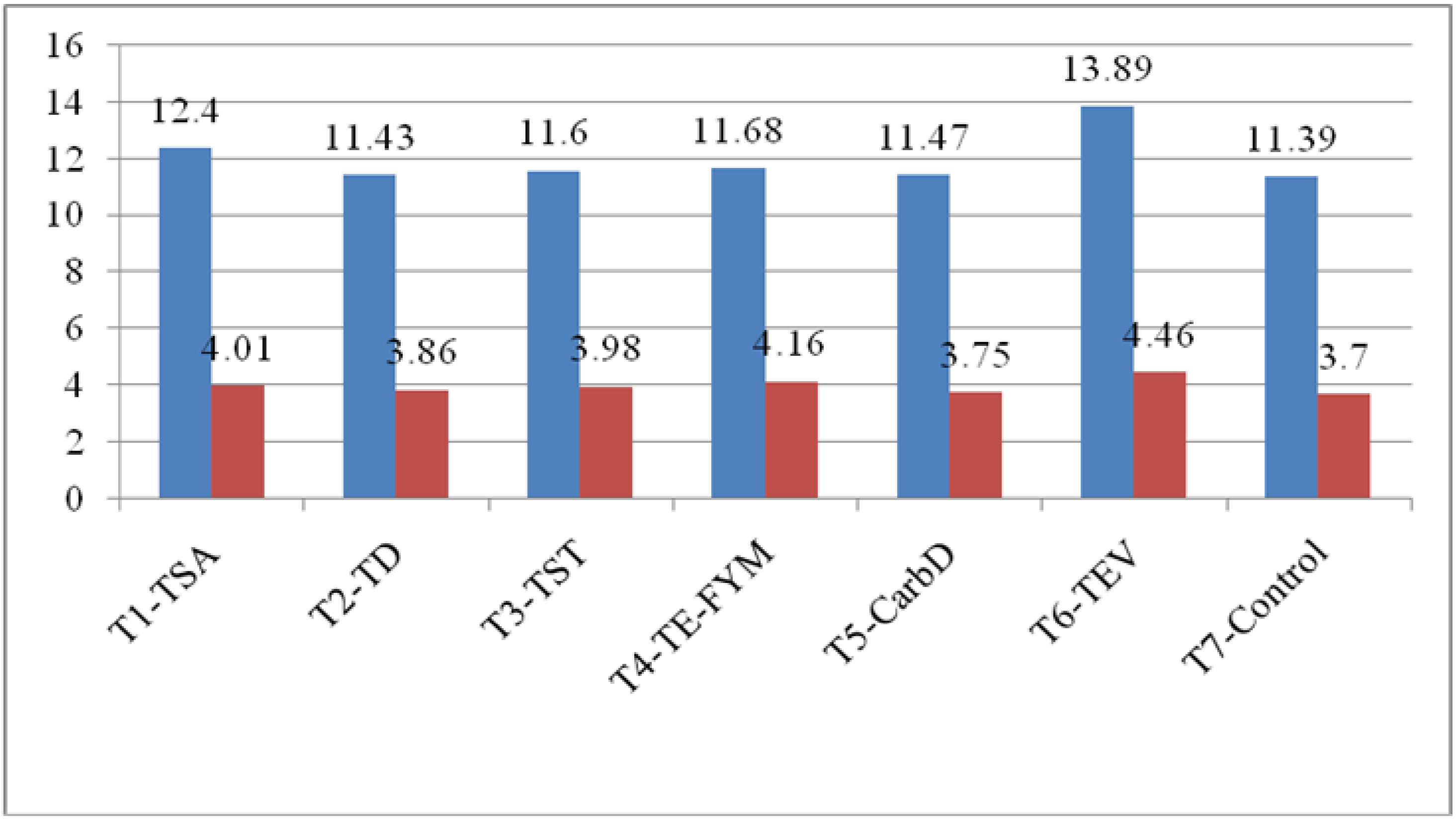


Figure 4

Effect of Trichoderma formulation on shoot and root growth of tomato in nursery (2019-20) Shoot length (cm) Root length (cm)



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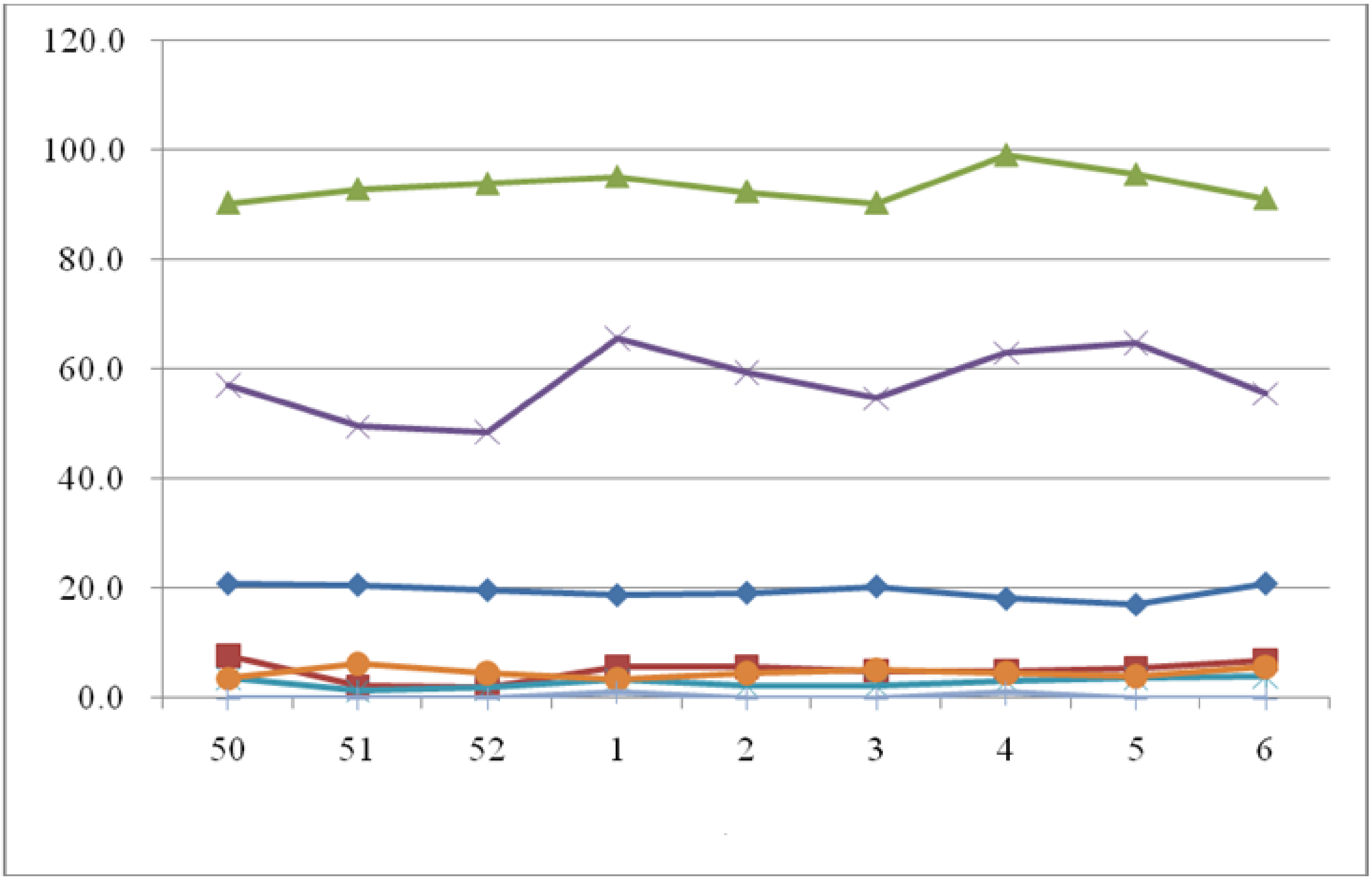

Figure 5

Weather conditions during first year (2018-19)

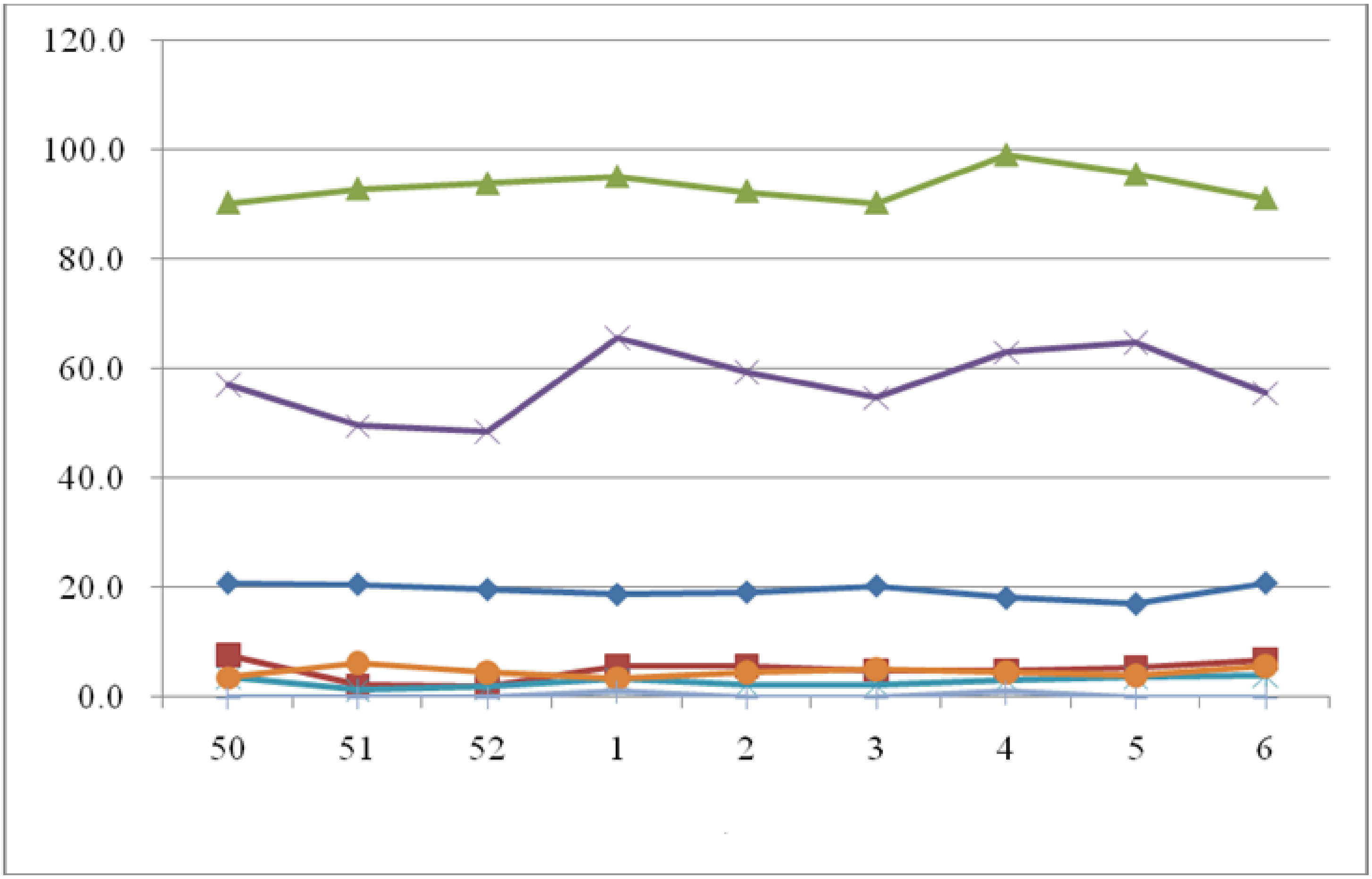

Figure 5

Weather conditions during first year (2018-19)

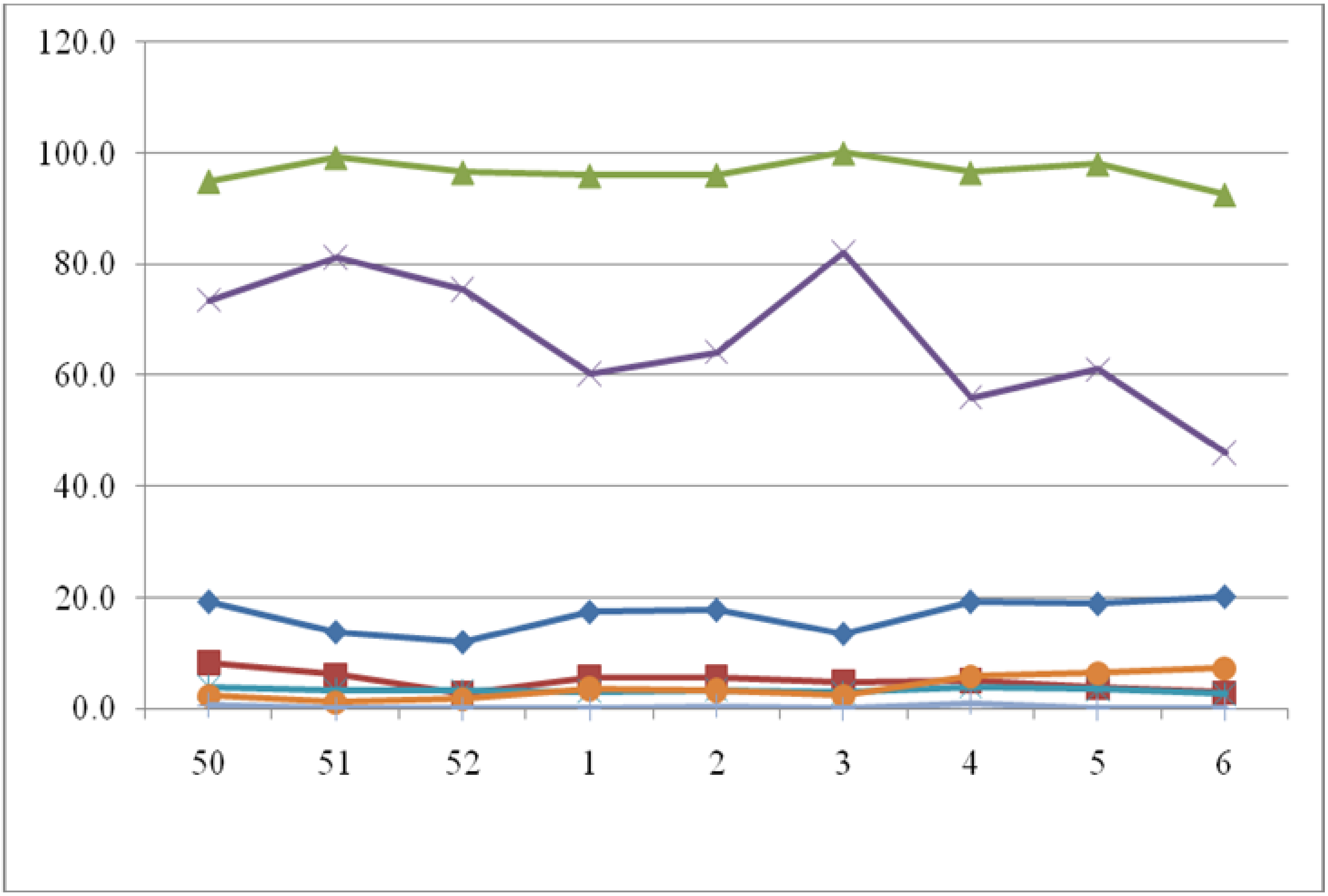

Figure 6

Weather condictions during second year (2019-20)

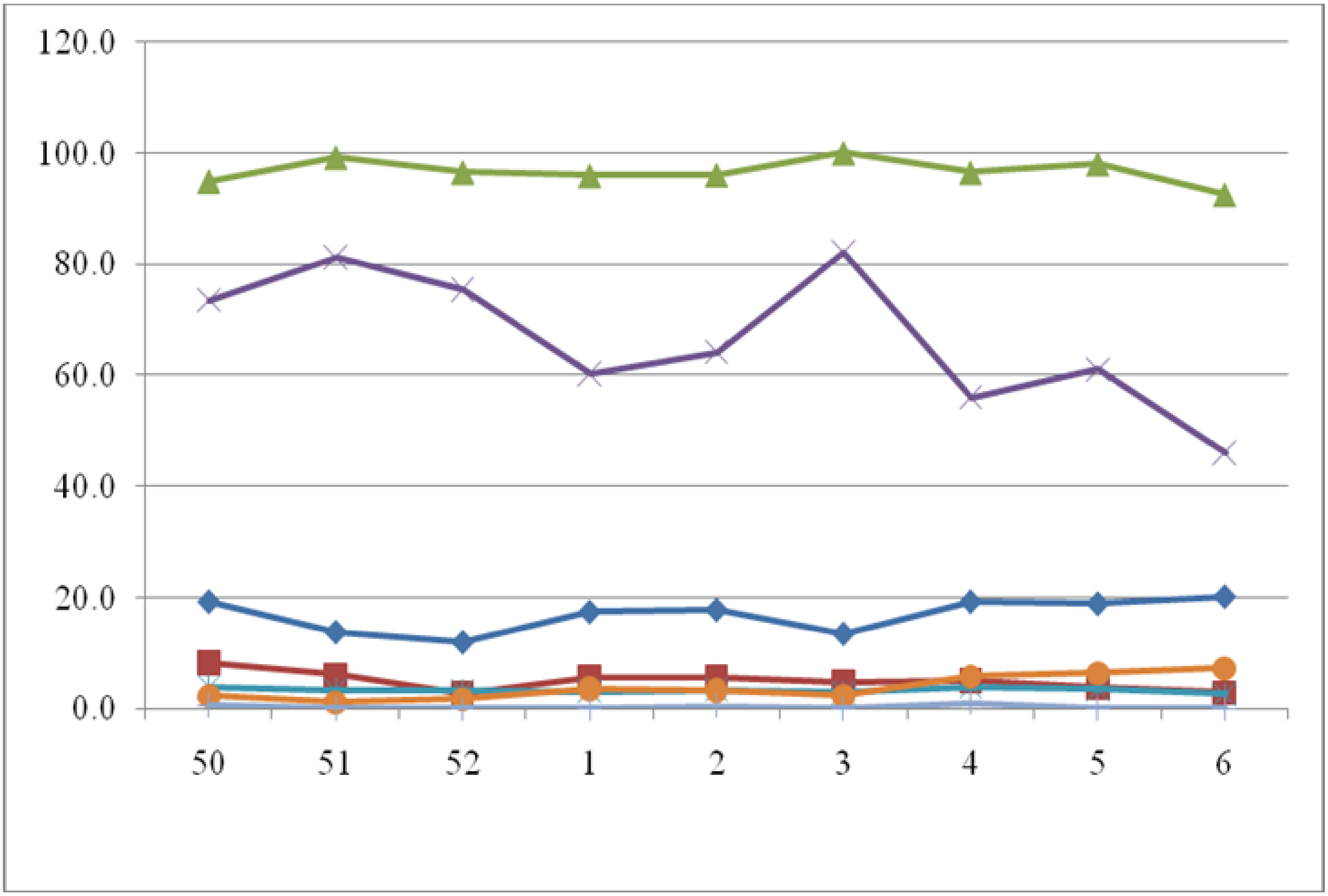

Figure 6

Weather condictions during second year (2019-20)