# Temperature mediated influence of mycotoxigenic fungi on the life cycle attributes of *Callosobruchus maculatus* F. in stored chickpea

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# Abstract

Environmental factors (biotic and abiotic) are a major source of grain reserve depletion. Fungi and insect pests both damage grains synergistically in the store. Fungal and insect pest infestations pose nutritional damage to the stored food which becomes unpalatable for the consumer. There is a need to develop a timeline for synergistic damage caused by insect pest and mycotoxigenic fungi to manage them in stored products. For this purpose, interaction of mycotoxigenic fungi (*Aspergillus flavus, Aspergillus niger, Penicillium digitatum* and *Alternaria alternata*) along with *C. maculatus* (Fabricius) (Bruchidae: Coleoptera) was studied at wide range of temperature. Development of *C. maculatus* was observed on fungus inoculated and non-inoculated *C. arietinum* seeds under different temperatures. *C. maculatus* was found attracted for completion of its life cycle attributes (Fecundity, larval emergence, pupae and adults) in fungus inoculated grains. Population of *C. maculatus* was decreased by increase in temperature but high temperatures pose to more fungi developments. Meanwhile, more egg laying was observed at 27 ºC and 33 ºC. At tested temperatures, larval emergence was high as compared other checked life attributes. Infestation of *A. flavus* and *A. niger was* also increased with different life stages of *C. maculatus* at all checked temperatures*. Penicillium digitatum* and *Alternaria alternata* infestation were increased in the *C. arietinum* at 27 ºC and 30 ºC respectively. This study will help in measuring the control practices of fungi and insect pest infestations in stored chick pea in Pakistan.


# 1. Introduction

Pakistan is fourth in chickpea (*Cicer arietinum* Linnaeus) production. It has high carbohydrate contents (62.34%) (El-adawy, 2002) and protein levels (23.67%) (Alajaji and El-Adawy, 2006; FAO, 2018). Pakistan faces considerable losses (15–55%) in chickpea crops during storage (Vanzetti et al., 2017). Contamination of stored commodities is mostly reported with microflora and insect infestations (Bhat, 1988; Delouche, 1980; Mills, 1986; Tuda, 1996). Mycotoxins are non-volatile secondary metabolites, produced by filamentous fungi which reduce the quality of stored food by damaging their physical appearance and chemical composition (Bräse et al., 2009). Mycotoxins mediated semiochemicals are considered as an indicator of rotten odor in grains and simulate interaction among insects and fungus species (Bennett and Inamdar, 2015; Bennett et al., 2012)

In stored commodities, the species of genus *Aspergillus* and *Penicillium* more proliferate due to high relative humidity and mycotoxins (Dawar et al., 2007; Kumar et al., 2009; Patil et al., 2012; Shukla et al., 2012). *Aspergillus flavus* contributed 64% of more aflatoxin production in *C. arietinum* seeds as compared to quality-added products of chickpea (Ramirez et al., 2018). There have been deleterious consequences of chick pea consumption contaminated with toxigenic fungi on human health and animals (Urooj et al., 2015).

The granivorous *Callosobruchus maculatus* F. (cowpea weevil)(Coleoptera: Chrysomelidae: Bruchidae) is the considerable causative agent of severe losses in seed germination, weight and nutritional level of legumes (Généfol et al., 2018; Staneva, 1982; Valencia et al., 1986). The *C. maculatus* can destroy dry

beans in tropical and arid climatic zones, especially in stores (Tuda et al., 2006). Beetles can develop in the availability of reduced water and food quantity in storage ecosystems (Dongre et al., 1996). The penetration of storage fungi in stored commodities occurs due to the mishandling after harvest, presence of dust residues, cracks in seed coat because of mechanical handling and insects (Woloshuk and Martínez, 2012).

Temperature is also a fundamental aspect related to insect physiology (Ratte, 1984) and biochemistry (Downer and Kallapur, 1981). The various ranges of temperature affect the survival of Bruchidae species and insect activities (Giga and Smith, 1987; Miyatake et al., 2008; Soares et al., 2015). Development of bruchid beetles is highly responsive to ranges of temperature which are also responsible for fungal communities development in post-harvest practices (Kistler, 1995; Sautour et al., 2001; Umoetok Akpassam et al., 2017). The well-studied temperature variables for all pathogenic microbes are 15–37°C. The optimum temperature for growth of *A. flavus* is 37°C, while *Penicillium* species are also developed at lower temperatures i.e., from room temperature to 0°C (Asurmendi et al., 2015; Lahouar et al., 2016; Palou, 2014).

Current study is designed to interpret the relationship between fungi species (*Penicillium digitatum, Aspergillus flavus, Aspergillus niger, Alternaria alternata*) and population builup of *C. maculatus* in stored grains at different temperature ranges(25°C, 27°C, 33°C and 35°C) at constant humidity (70%). Current findings will be helpful in developing some IPM strategy for *C. maculatus* and fungal infestation in stored products

## 2. Materials And Methods

# 2.1. Insect culture

*C. maculatus* was cultured on presanitized chickpea grains under constant environmental conditions (25 ± 5°C and 55 ± 5% R.H.) to obtain a uniform population. Males and females were separated by using protocols of (Beck and Blumer, 2011).

# 2.2. Collection of chickpea

For experiment, we collected stored chickpea (kabuli variety) (not freshly harvested) from the retailors of four different selected locations (Fig. 1): Dg Khan, Lodhran, Muzaffargarh, and Multan kept at 25 ± 5°C and 55 ± 5% R.H. in Ecotoxicology Laboratory, Department of Entomology, Bahauddin Zakariya University (BZU) Multan, Pakistan (+ 30° 11' 52"N, + 71° 28' 11" E). All the samples were stored in autoclaved glass jars (round cylinderrical with dimensions of 32 x 23 x 23 cm) covered with aluminum foil and transferred directly to the mycological laboratory for fungus infestation via PDA (Potato Dextrose Agar)

# 2.3. Isolation of mycotoxigenic fungi

Isolation of fungi from *C. maculatus* and *C. arietinum* was performed in fungi research laboratory, Department of Plant Pathology, Bahauddin Zakariya University, Multan, Pakistan. Seven *C. maculatus*

were made sterile with 2% solution of sodium hypochlorite, washed twice with distilled water, dried on blotter paper, crushed and placed on PDA (Potato Dextrose Agar) plate.The PDA plates were prepared by Potato 125 g, Dextrose 10 g and Agar 7.5 g in 500 ml distilled water. Five healthy-appearing grains of *C. arietinum* were collected from the stock. Grains were disinfected with 2% sodium hypochlorite, washed with distilled water twice, dried on blotting paper and placed on PDA plates for fungal growth observation (Bosly and Kawanna, 2014; Taylor and Sinha, 2009). All the isolation procedures were done in a horizontal laminar flow cabinet (Airstream® (LHG) (Lamboni and Hell, 2009). The isolation procedure for both insects and grains were replicated 10 times.

## 2.4. Identification of mycotoxigenic fungi

Identification of different fungi spp. performed at Plant Pathology Department of Bahuddin Zakariya University (BZU) Multan, Pakistan. Mycological evaluation through microscopic examination was done by staining the hyphae with methylene blue on glass slides from fresh fungi cultures (Morishita and Sei, 2006).

## 2.5. Purification of mycotoxigenic fungi

Purification was done to remove contamination of other microbes from fungi cultures. Stainless steel needles were used for the purification of fungi culture. Sterilized the needle on the spirit lamp and then needles were used to separate the piece of PDA containing required fungal species. Placed fungal spores on PDA plate in an incubator at 25°C and observed daily to check the culture purification (Ko et al., 2001).

## 2.6. Percentage of fungus

The rate of occurrence of specific fungus species in an isolated culture of insect and grains was determined by the following formula (Eq. 1) (Ahmad and Singh, 1991):

$$\mathrm{FrequencyofFungus}(\%) = \frac{\mathrm{Totalno.ofseedscontainingparticularfungus}}{\mathrm{Totalno.ofseedsused}} \text{ X } 100$$

## 2.7. Spore suspension

Fungi (*A. flavus, A. niger, P. digitatum* and *A. alternata*) isolated from *C. arietinum* and *C. maculatus* were used for spore suspension. The suspensions were prepared from 7 days of fresh cultures of fungus. Fungal spoers of mycotoxigenic fungi species were scraped with the help of glass slide by adding 20 ml autoclaved distilled water, and then solution was stirred in magnetic stirrer until conidia become separated from PDA. After agitation impurities of suspension were removed by filtering it through filter paper. The numbers of spores were counted under a light microscope through haemocytometer.

## 2.8. Inoculation of *C. arietinum* grains

Spore suspension was diluted to obtain 5 X $10^3$ spores/ml to inoculate *C. arietinum*. Autoclaved chickpea grains were inoculated with four different fungi by adding 3 ml of spore suspension per 100 g grains in glass jar (h = 5",W = 3.2") (Nesci and Montemarani, 2011).

## 2.9. Influence of temperature on growth of C. maculatus and mycotxigenic fungi

The normal prevailing temperature ranges (27, 30, 33 and 35°C) were tested for the growth of *C. maculatus* and mycotoxigenic fungi simultaneously, at 70% R.H. (Gillooly et al., 2002). All the selected temperatures and humidity were maintained in Laboratory climate chamber (MLR-352-PE).

## 2.10. Extent of fungi on life cycle of *C. maculatus*

Five pairs of surface-sterilized (2% sodium hypochlorite) adults were introduced into glass jar (Height = 5",width = 3.2") each containing 100 g inoculated *C. arietinum* in Ecotoxicology Laboratory, Department of Entomology, Bahauddin Zakariya University, Multan and removed after 24 hours (Tsai et al., 2007). All the experimental units were maintained in a growth chamber with four constant temperatures 27°C, 30°C, 33°C & 35°C and R.H. 70%. *Cicer arietinum* were checked for numbers of *C. maculatus* eggs, larvae, pupae and adults by dissecting grains along with growth of fungal species (Howe and Currie, 1964). Each treatment was replicated 4 times.

## 2.11. Statistical analysis

Incidence (%) of fungal species in *C. maculatus* adults and chickpea grains were analyzed via frequency equation. While, Chi square test and Two-Way ANOVA of the fungal isolation frequency were performed by subjecting data to a computational basaed software SPSS (SPSS VERSION 7.0). Variables (temperature, humidity and number of days) were compared through Duncans Multiple Range Test (DMRT) at 95%. Developmental activity of *C. maculatus* (dependent variable) correlated with constant humidity (70%), selected temperatures and fungal species (considered as independent variables) was calculated through software (SAS Institute, 2000).

# 3. Results

## 3.1. Isolation of mycotoxigenic Fungi

### 3.1.1. From *C. arietinum* grains

*C. arietinum* grains were analyzed for fungal colonies, *Aspergillus, Fusarium, Penicillium* and *Alternaria* were prominent as seed spoilers. *A. flavus, A. niger, A. alternata, P. digitatum* and *F. oxysporum* were examined as dominant species from all localities sampled. *C. arietinum* was found to be highly infested with *A. flavus* (52.3) while *A. alternata* had the lowest frequency of colonies (8) among the four tested fungal species. Colonies of *A. niger, P. digitatum* and *F. oxysporum*, were found statistically similar, while *A. flavus* and *A. alternata* had a significant difference ($F = 32.009$; $df = 4(20)$; $P < 0.001$) between fungal colony frequencies (Table 1).

Table 1
Mycotoxigenic fungi isolated from *C. arietinum* samples purchased from different locations of Punjab Pakistan.

| Species | Multan (n = 75) | Muzaffargarh (n = 75) | Lodhran (n = 75) | DG khan (n = 75) | Total Isolates (n = 300) | Isolation Frequency SE(±) |
|---|---|---|---|---|---|---|
| *A. flavus* | 72 | 28 | 27 | 30 | 157 | 52.3 ± 14.58a |
| *A. niger* | 33 | 15 | 21 | 13 | 82 | 27.3 ± 6.00b |
| *A.alternata* | 10 | 3 | 7 | 4 | 24 | 8 ± 2.11b |
| *F. oxysporum* | 30 | 8 | 7 | 19 | 64 | 21.33 ± 7.20b |
| *P. digitatum* | 30 | 11 | 11 | 14 | 66 | 22 ± 6.07b |
| Means within a column followed by the same letter are not significantly different from each other (SPSS software at 0.05). | | | | | | |

## 3.1.2. From *C. maculatus*

Fungal growth observation from the body of *C. maculatus* revealed that *A. flavus* was found frequently (71.43) while *A. alternata* was the minimal fungal isolate from both adult stages was 4.64 (Table 2). Diversities of fungus are present on the body of *C. maculatus* but there is no association between gender of insect and types of fungus they are independent ( $X^2 = 6.339^a$; df = 4 ; P > 0.175)

Table 2
Isolation of fungi from both male and female adults of *C. maculatus.*

| Species | No. of isolates | | | Isolation Frequency |
|---|---|---|---|---|
| | Female (n = 140) | Male (n = 140) | Total isolates (n = 280) | |
| *A. flavus* | 98 | 102 | 200 | 71.43 |
| *A. niger* | 20 | 24 | 44 | 15.71 |
| *A. alternata* | 3 | 10 | 13 | 4.64 |
| *F. oxysporum* | 24 | 37 | 61 | 21.79 |
| *P. digitatum* | 32 | 26 | 58 | 20.7 |
| (Chi square test at 0.05) | | | | |

# 3.2. Identification of mycotoxigenic fungi

Fungal species isolated from *C. arietinum* and *C. maculatus* were identified at Plant Pathology Department of Bahuddin Zakariya University (BZU) Multan.

## 3.4. Interaction among mycotoxigenic fungi and *C. maculatus* in *C. arietinum* grains at different temperatures

### 3.4.1. Developmental period of *C. maculatus*

*C. maculatus* were tested for their life cycle attributes (egg,larvae, pupae and adult stages) on inoculated *C. arietinum* grains at four different temperatures (27 ºC, 30 ºC, 33 ºC, 35 ºC). *C. maculatus* population was significantly developed at all tested temperatures ($F = 81.85$; $df = 3(60)$; $P < 0.0001$). As the temperature increased *C. maculatus* life period was shortened and at 35 ºC, the insect showed shortest life period. Meanwhile, intensification in temperature also increased the progress of *A. flavus* and *A. niger* in grains. The life period of *C. maculatus* was elapsed to 33 days (highest recorded days) at 30°C. Impact of Fungal growth was found non-significant with the life cycle attributes of *C. maculatus* ($F = 1.03$; $df = 4(60)$; $P = 0.4006$) and all tested temperatures ($F = 1.51$; $df = 12(60)$; $P = 0.146$) (Fig. 2).

### 3.4.2. Fecundity of *C. maculatus*

Oviposition rates of *C. maculatus* were significantly affected by all tested temperatures ($F = 564.37$; $df = 3(60)$; $P < 0.0001$). The females of *C. maculatus* preferred inoculated *C. arietinum* more than sterilized *C. arietinum* grains for oviposition at all temperatures. At 27°C and 33°C, oviposition was 403.75 on *C. arietinum* grains inoculated with *A. flavus* and *A. niger*. Reduction in the oviposition occurs at 35°C in inoculated and control *C. arietinum*. *P. digitatum* inoculated grains exhibited a few oviposition (33.25). Oviposition rate was highly significant with relation to fungal inoculation ($F = 192.23$; $df = 4(60)$; $P < 0.0001$) and temperature ($F = 83.05$; $df = 12(60)$; $P < 0.0001$) (Fig. 3).

### 3.4.3. Incubation period

Incubation period of *C. maculatus* were not significantly ($F = 0.40$; $df = 4(60)$; $P = 0.80$) influenced by interaction of fungi and temperature ($F = 0.66$; $df = 12(60)$; $P = 0.78$). Highest incubation period was of 8.25 days at 27°C and also same number of days were observed in presence of all tested fungal species on *C. arietinum*. Temperature ranges exhibited highly significant ($F = 35.93$; $df = 3(60)$; $P < 0.0001$) effect on the incubation period of *C. maculatus.* Increase in temperature was also decreased the incubation period of *C. maculatus* as observed in *P. digitatum* infested *C. arietinum* grains was shows shortest incubation period (3.5 days) at 35°C (Fig. 4).

### 3.4.4. Larval emergence of *C. maculatus*

Larvae of *C. maculatus* were highly influenced due to temperatures ($F = 98.15$; $df = 3(60)$; $P < 0.0001$) and mycotoxigenic fungi ($F = 104.49$; $df = 4(60)$; $P < 0.0001$) infestation in *C. arietinum.* Larvae emergence was lower than oviposition of *C. maculatus* in inoculated *C. arietinum*. Fungal prevalence in grains at different temperatures was decreased the larvae emergence. Moreover, opposite results for larvae emergence were observed in instances of non-inoculated (323 at 27°C) and *A. niger* inoculated (275.5 at 33°C) *C. arietinum*. The successive highest rate of larvae emergence was evaluated in *C. arietinum* infested with *A. niger*. Numbers of larvae emergence was also significantly affected because of

interaction among fungi and temperatures (F = 19.38; df = 12(60); P < 0.0001). The results also indicated the lowest number of larvae emergence in *P. digitatum* infested *C. arietinum* at 35°C was 33.25 (Fig. 5).

### 3.4.5. Pupation of *C. maculatus*

The mycotoxigenic fungi (F = 153.97; df = 4(60); P < 0.0001), temperatures (F = 158.96; df = 3(60); P < 0.0001) and their interaction (F = 38.90; df = 12(60); P < 0.0001) exhibited highly significant effects towards pupation rate of *C. maculatus.* However, *C. arietinum* infested with *P. digitatum* and *A. alternata* shows the lowest pupation rate was 28.5 and 38 at 35°C. High numbers of pupae emerged at 27°C in inoculated and un-inoculated *C. arietinum* as compared to other temperatures and control (Fig. 6).

### 3.4.6. Adult emergence

Adult emergence of *C. maculatus* were inversely correlated with fluctuating temperatures (F = 202.02; df = 3(60); P < 0.0001), mycotoxigenic fungi (F = 97.95; df = 4(60); P < 0.0001) (F = 18.30; df = 4(60); P < 0.0001) and their interactions (F = 17.37; df = 12(60); P < 0.0001). Results demonstrated that, at 27°C maximum adults were emerged in inoculated and non-inoculated *C. arietinum.* However, the lowest adult emergence rate at 35°C in *P. digitatum* inoculated *C. arietinum* was 23 (Fig. 7).

## 4. Discussion

Six-month storage of *C. arietinum* grains in different storage conditions face deterioration (26%) and weight loss (55.67%) because of *C. maculatus* infestation (Babu et al., 2020). *C. arietinum* with thin seed coat, large seed size, high carbohydrates and phenols were more susceptible to *C. maculatus* infestation (Swamy et al., 2020). Adults of *C. maculatus* enclosed mycotoxigenic fungi (*Aspergillus, Penicillium, Fusarium* and *Alternaria)* species inside the body, as previously suggested by (Kumari et al., 2011) in case of red flour beetle. In this study we observed that the proliferation of mycotoxigenic fungi in *C. arietinum* also increased due to *C. maculatus* infestation within storage ecosystem. The presence of both *C. maculatus* and mycotoxigenic fungi species significantly takes part in the deterioration of stored commodities (Allotey et al., 2010). Results revealed high frequencies of *A. flavus* and *A. niger* isolates from *C. maculatus* adults. On the other hand, similar results reported that infestation of mycotoxigenic fungi in *Triticum aestivum* observed beacuase of *T. castaneum* and *Sitophilus granarius* activities (Agrawal et al., 1957; Bosly and Kawanna, 2014). The presence of mycotoxigenic fungi in an insect's body illustrates that insects were able to transfer fungal flora in grains. Red flour beetle had been associated with dissemination of mycotoxigenic fungi to their hosts (Bosly and Kawanna, 2014) and also observed in stored rice grains (Yun et al., 2018). *C. maculatus* larvae, pupae and adults were significantly affected by the infestation of mycotoxigenic fungi.

Insect pests have intrinsic ability to develop and reproduce to change in temperature and time progressively (Burges, 2008). Temperature is inversely interacting with growth rate of insects. Resuts explain that *C. maculatus* completed its life cycle on all tested fungus and temperature parameters. The progressive period of *C. maculatus* was shorter on all infested and non-infested *C. arietinum* at 35°C and 70% R.H. Shortest life period of *Cadra cautella* were demonstrated at 30°C and 70% R.H. (Burges and

Haskins, 1965). *Ahasverus advena* also complete their life cycle on different concentrations of Aflatoxin $B_1$ infested grains and the shortest life cycle was observed at 30°C (Jacob, 1996; Zhao et al., 2018). Emergence of larvae was high at 27°C on sterilized *C. arietinum* as well as on *C. arietinum* infested with *A. niger* at 33°C. Similar results were observed in development of *Trogoderma granarium* on broken wheat grains at 35°C while maximum fecundity and larvae emergence was evaluated at 30°C (Riaz et al., 2014). The infestation of mycotoxigenic fungi was able to influence the development period of *C. maculatus* in stored grains under selected temperature ranges.

Longest development period of *C. maculatus* was analyzed 33 days at 30°C and more than 25 days at 27°C on inoculated and non-inoculated *C. arietinum*. *C. maculatus* showed an incubation period of more than 6 days at 30°C on all tested parameters. This is higher than other pests including *Chilo partellus* was showed shortest incubation period (4 days) at 30°C but a similar development period of more than 30 days was observed at 30°C at 80% R.H. (Tamiru et al., 2012). Maximum progress rate of *A. flavus* was noticed at 35°C (Mannaa and Kim, 2018). *A. alternata* shows maximum growth rate at 25°C on *Glycine max* (Oviedo et al., 2011). Meanwhile germination of *Penicillium* spp. was observed at 30°C at 75% humidity (Pasanen et al., 1991). *Fusarium* spp. was not showed any germination at temperature up to 25°C on *G. max* medium (Garcia et al., 2012) that's why we do not select this species as the medium for *C. maculatus* growth on these temperature ranges.

Temperature influenced the fecundity of *C. maculatus* more than humidity. Temperature and suitable host preferences are the most considerable factors related to the development and oviposition of *C. maculatus* (Giga and Smith, 1987; Mam and Mohamed, 2015). The results presented maximum oviposition and larvae rate of *C. maculatus* on *A. flavus* inoculated *C. arietinum* at 27°C as well as on *A. niger* at 33°C. High numbers of larvae, pupae and adults were observed on sterilized *C. arietinum*. *C. maculatus* preferred *A. flavus* and *A. niger* infested *C. arietinum* more than control for oviposition. This is in contrast to; Corns infested with *A. halophilicus* were more suitable for the oviposition of *P. interpunctella* while a high development rate was observed at autoclaved corn (Abdel-Rahman H. A., 1969). *T. stercorea* showed minimum oviposition and maximum numbers of larvae on *A. flavus* at 30°C. Average oviposition rate of *C. maculatus* on the *P. digitatum* (33–209) was highest as compared to *T. stercorea* was lowest on *P. purpurogenum* (42). Minimum larvae emergence was observed in *T. stercorea* on *P. purpurogenum* (Jacob, 1988; Tsai et al., 2007) as the same results were also observed for *C. maculatus* on *P. digitatum* *C. arietinum*.

*C. maculatus* preferred to oviposit at 30°C and 35°C while maximum oviposition was observed at 30°C on sterilized *C. arietinum* (Chandrantha et al., 1987; Lale and Vidal, 2003). Researchers also found that *A. advena* and *Cryptolestes ferrugineus* were not able to oviposit on the *A. flavus* and *A. niger* isolates as observed in case of *C. maculatus* (David, 1974; Loschiavo and Sinha, 1966).

# 5. Conclusion

More than 70% of *C. arietinum* deteriorates because of mycotoxigenic fungi and *C. maculatus* in houses, markets and stores. All the identified fungi species are well known to produce mycotoxins and reduction in the nutritional value of *C. arietinum*. A reduced amount of fungal growth on non-infested autoclaved chickpea grains (control) was observed at all selected temperatures even in presence of weevil. Preventive measures for both pests should be applied at commercial levels on the tested temperatures ranges in stores, houses and markets.

1. *C. maculatus* was able to reproduce in both inoculated and non-inoculated grains on all selected temperatures.
2. Market stored chickpea grains were found with more than 50% frequency of mycotoxigenic fungi while this percentage increased to 70% when insects come in contact with the grains in storages
3. *C. maculatus* carr fungus in body and the relationship between both developed early at 35°C causing high quality damage of *C. arietinum*.

# Declarations


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### Competing interest

Authors declare no competing interest.

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# Figures

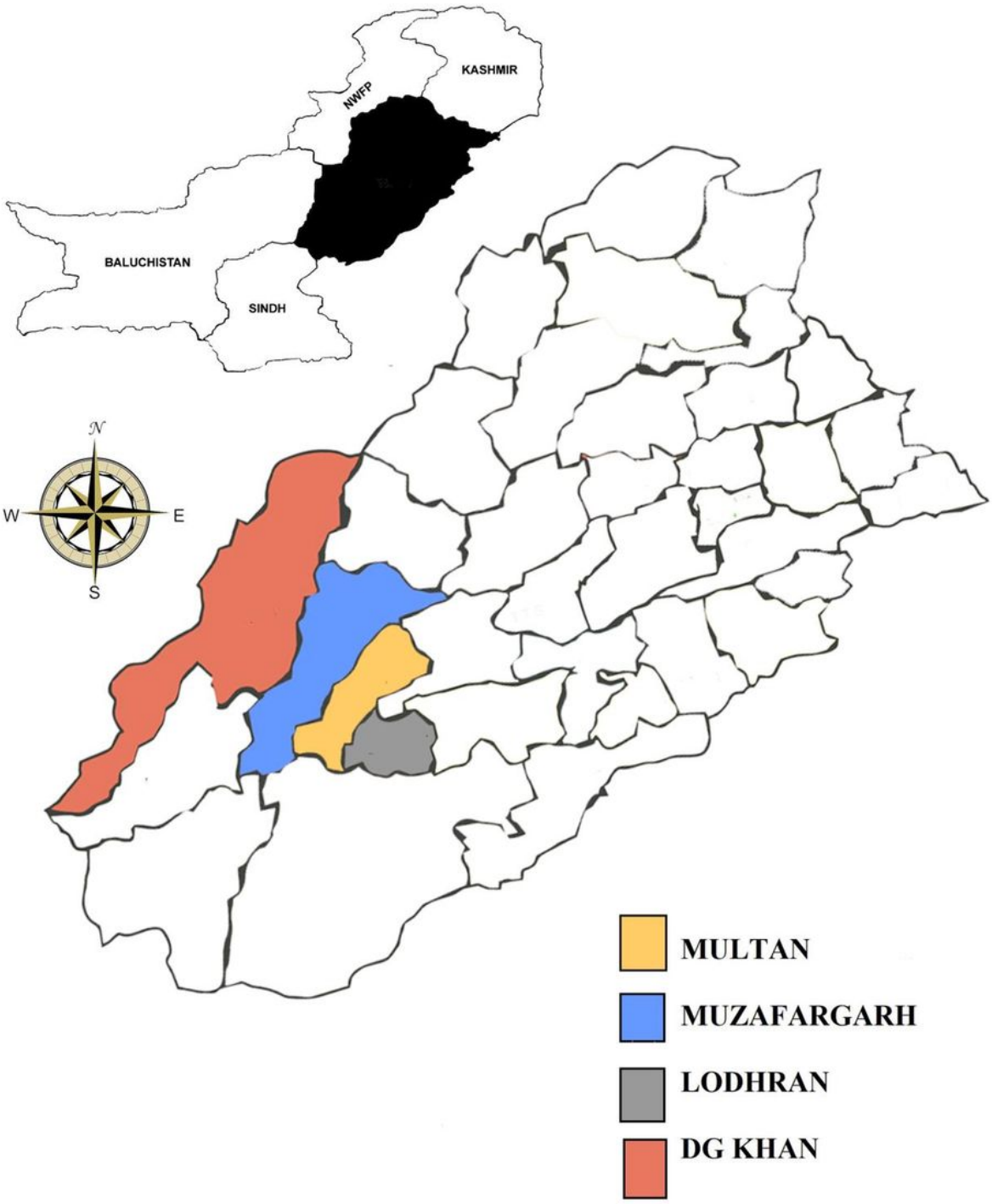
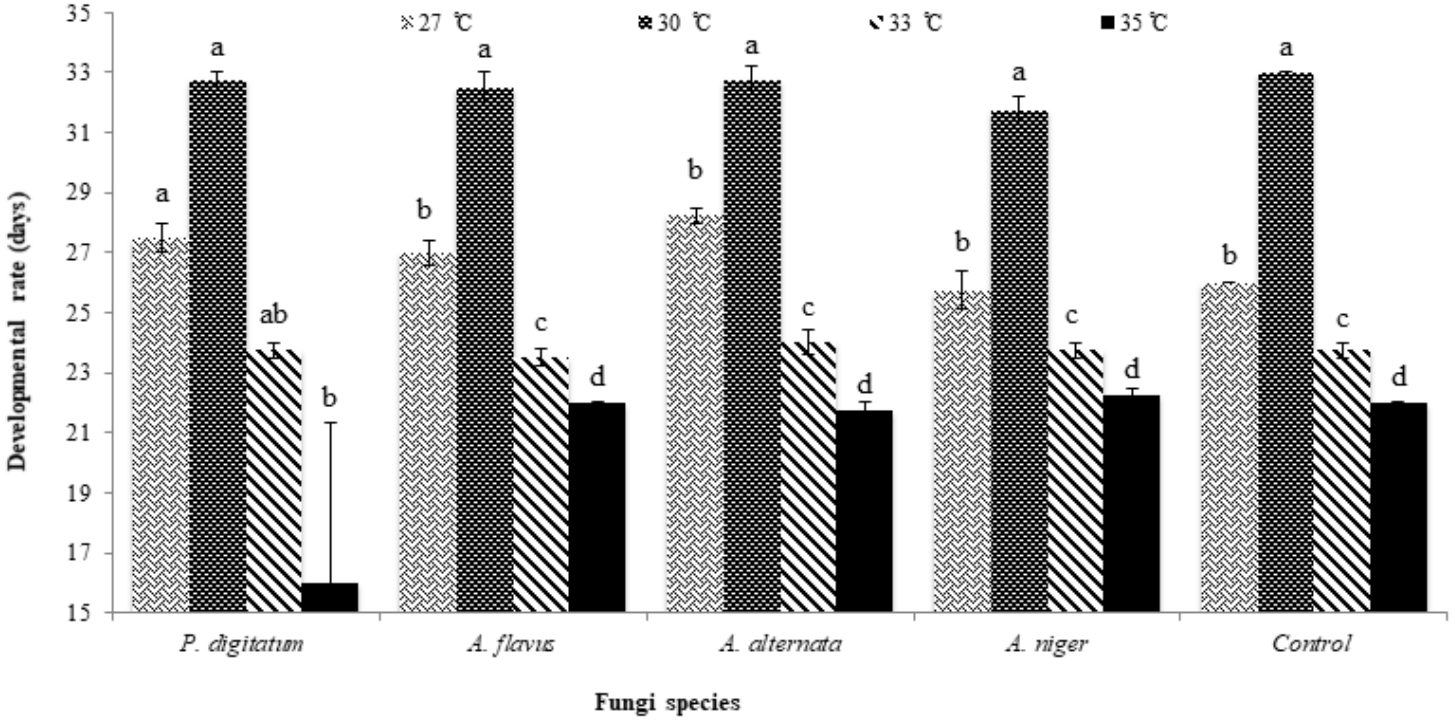


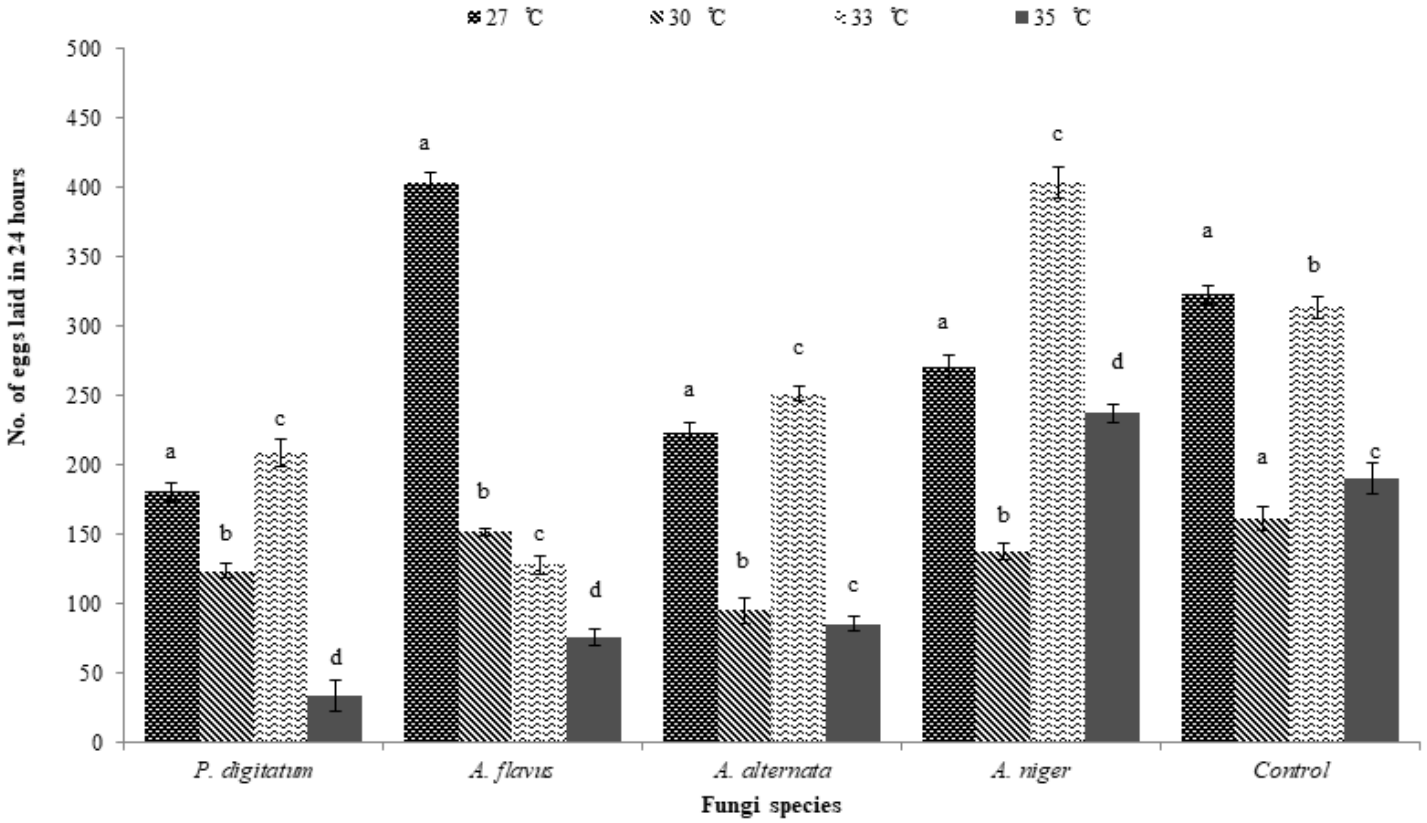

**Figure 1**

Locations for *C. arietinum* sampling in Punjab Pakistan.



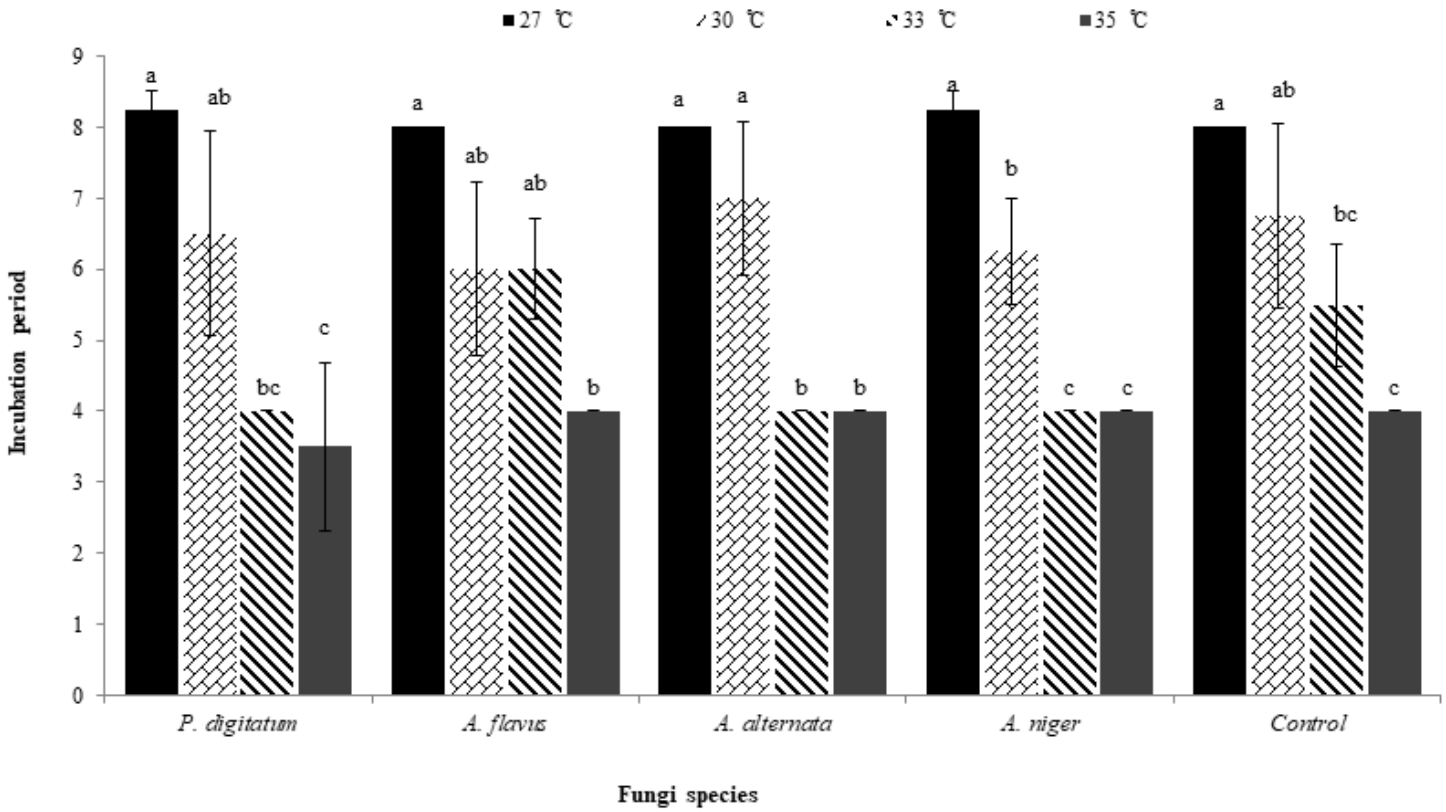

## Figure 2

*C. maculatus* development rate on fungal infected and non-infected *C. arietinum* at different temperatures. Error bars denote standard error (±SE).Duncan test at 0.05. (X- axis donates the effect of fungus species, Y- axis donates the life attributes of insects & Bars donates the effect of temeporature).



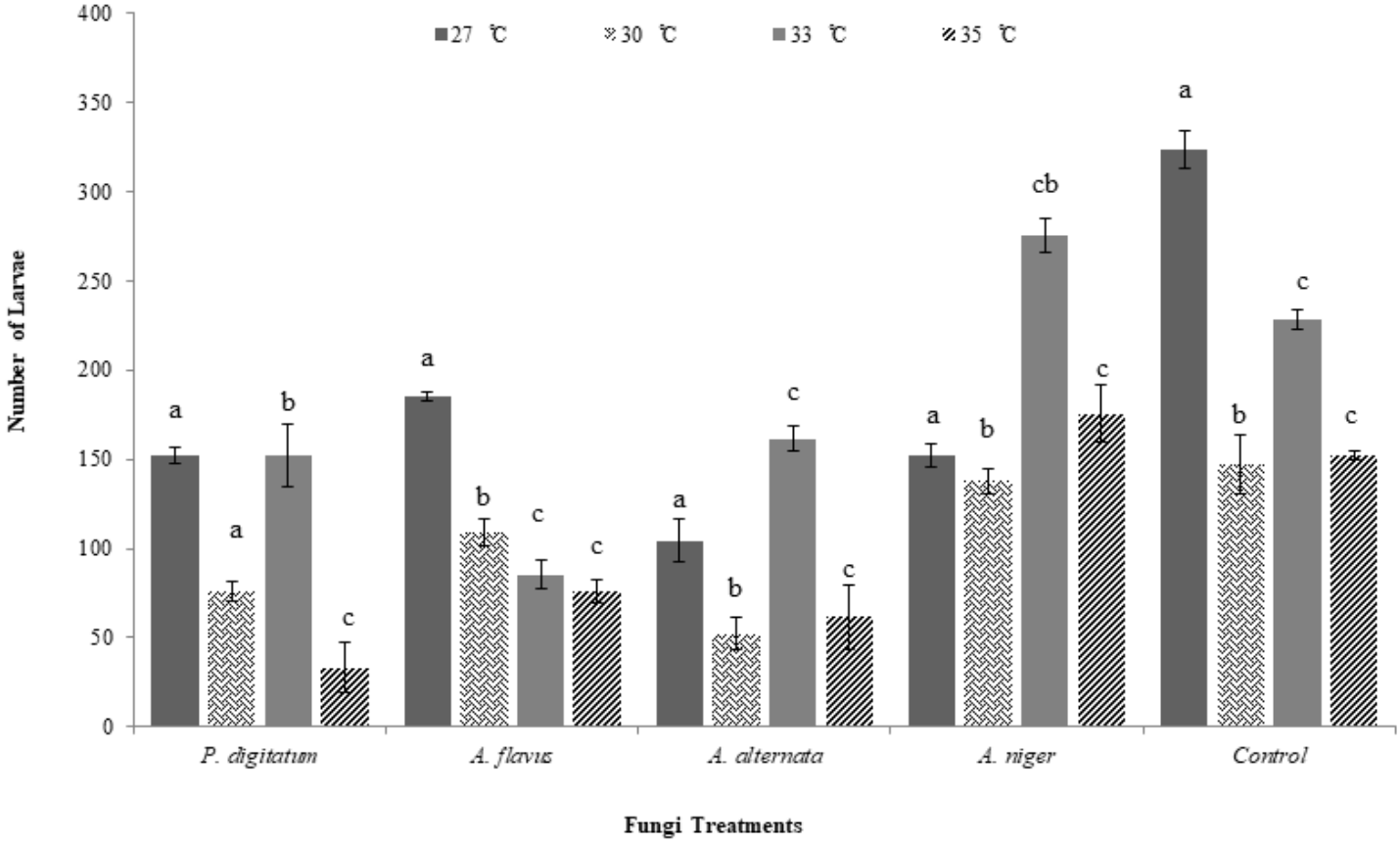

## Figure 3

*C. maculatus* egg laying on fungal infected and non-infected *C. arietinum* at different temperatures. Error bars denote standard error (±SE).Duncan test at 0.05.



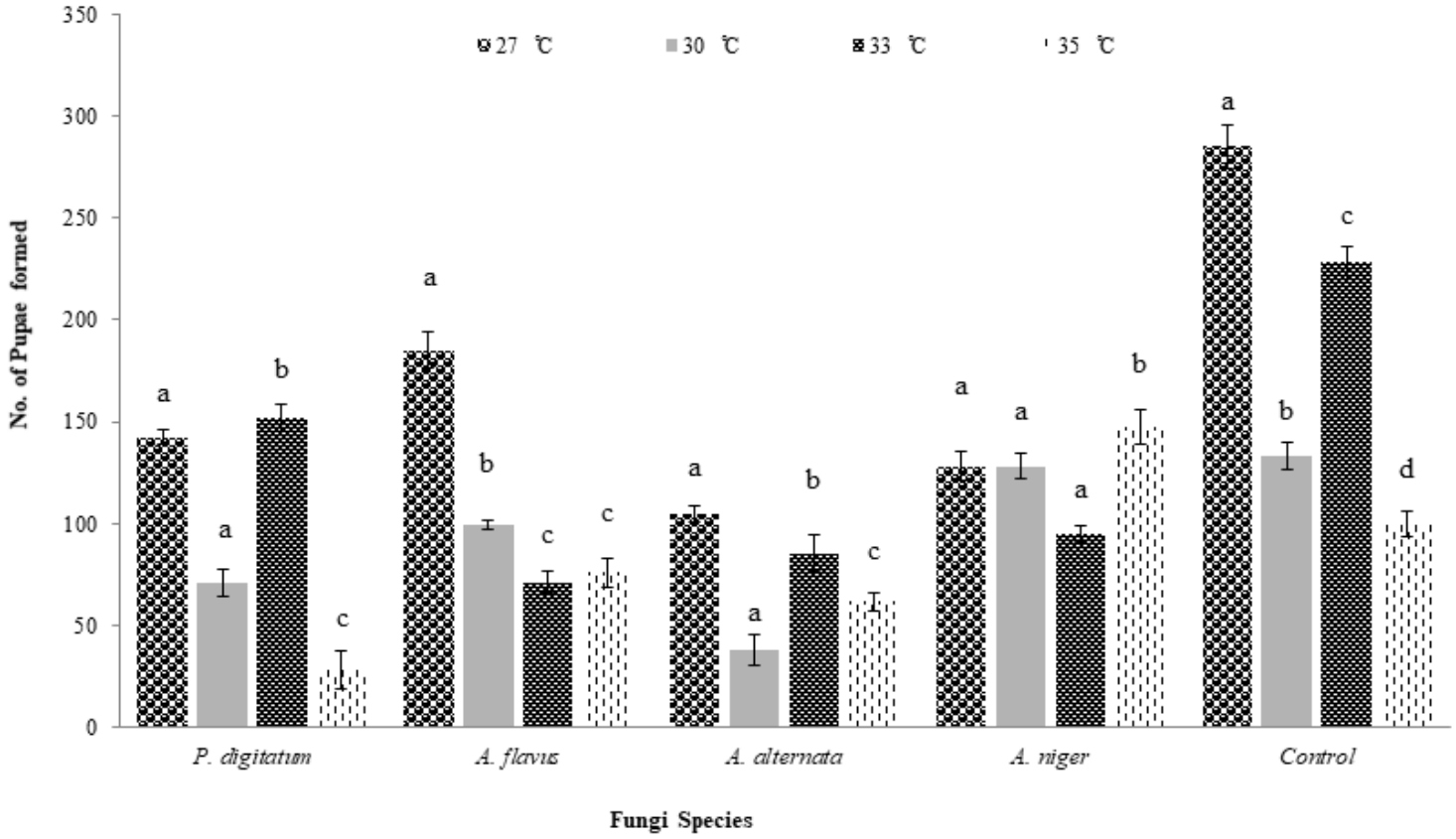

## Figure 4

*C. maculatus* incubation period on fungal infected and non-infected *C. arietinum* at different temperatures. Error bars denote standard error (±SE).Duncan test at 0.05.



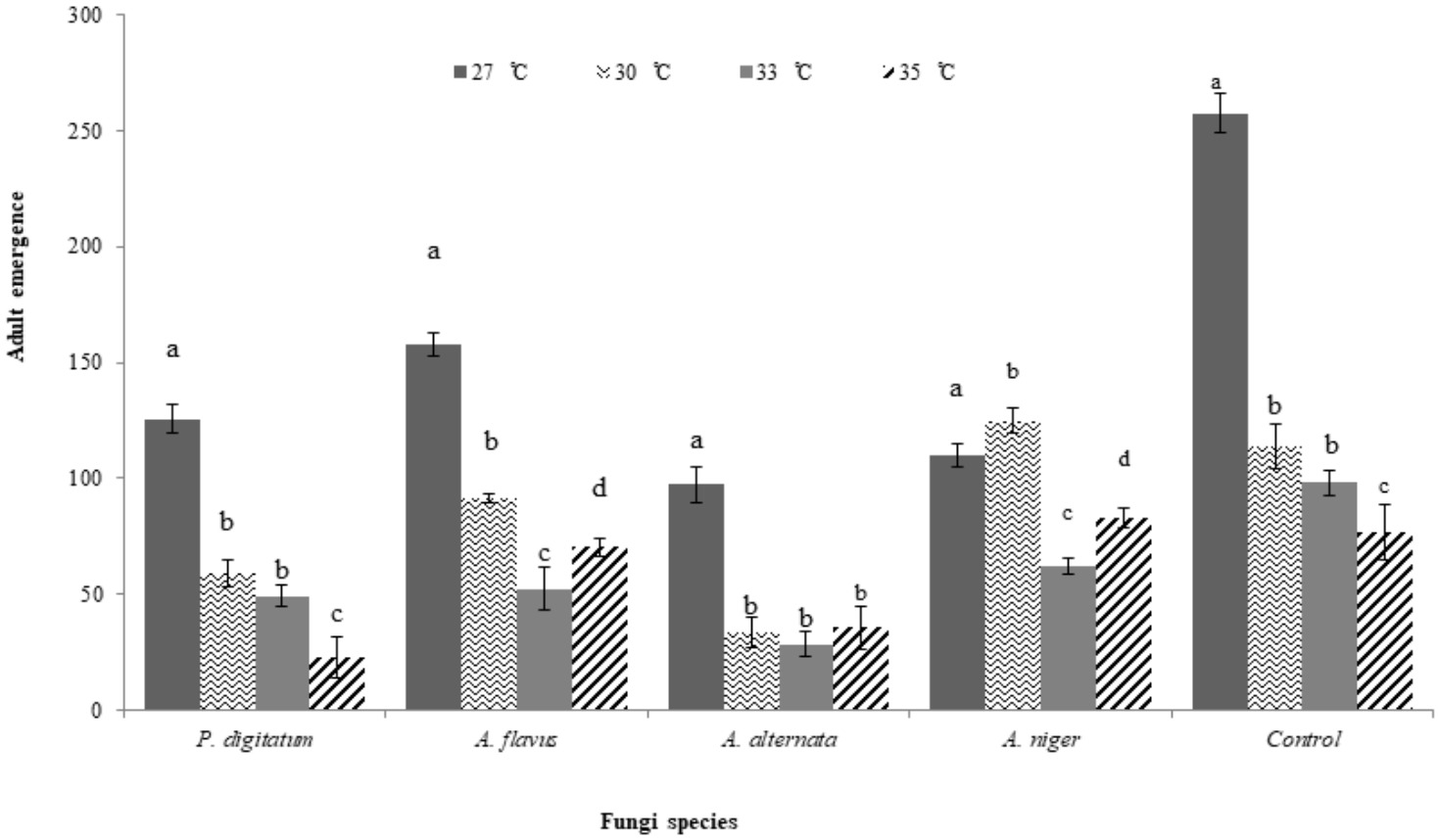

## Figure 5

Response of *C. maculatus* (larval population) on fungal infected and non-infected *C. arietinum* at different temperatures. Error bars denote standard error (±SE).Duncan test at 0.05.




## Figure 6

Response of *C. maculatus* (pupal population) on fungal infected and non-infected *C. arietinum* at different temperatures. Error bars denote standard error (±SE).Duncan test at 0.05.




## Figure 7

Response of *C. maculatus* (adult emergence) on fungal infected and non-infected *C. arietinum* at different temperatures. Error bars denote standard error (±SE).Duncan test at 0.05.