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SCREENING OF MEDICINAL PLANT EXTRACTS AGAINST *BEAUVERIA BASSIANA* INFECTION TO VTH INSTAR LARVAE OF *BOMBYX MORI* L.

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ABSTRACT

Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. The aqueous extract of *Azadirachta indica, parthenium hysterophorus*, and *Ocimum sanctum and pongamia pinnata* were tested for antifungal activity *in vitro*. The aim of study was to find out the effect of aqueous extracts on *Beauveria bassiana* infected *Bombyx mori* L. larvae. The aqueous extract of *A. Mexicana* shows effective results as compared to other botanicals. However it is clear that aqueous plant extracts showed decreased mortality with effective rate of rearing.

KEYWORDS: *Bombyx mori, Beauveria bassiana, Azadirachta indica, Parthenium hysterophorus, Osimum sanctum and Pongamia pinnata.*

INTRODUCTION

Many Indian plants have been used from time immemorial to treat various diseases and infections in traditional medicinal system of India such as Siddha, Ayurvedha and Nataropathy (Shekhawat and Prasad, 1971; Khanna and Chandra, 1972). Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti et al., 2008). Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicine, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs. Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design (Ebi and Ofoefule, 2000). The traditional method of extraction involves a number of solvents that are used in various cases (Ganesan and Krishnaraju, 1995). Among the fungal disease of silkworm, white muscardine is found to be serious caused by *Beauveria bassiana* (Bassi, 1835). Cocoon crop loss in different contries of about 5 to 50% reported by Jhansi Lakshmi (2003).

A wide variety of plant secondary metabolites have been identified as active principles for the treatment of various ailments (Taylor et al., 2001; Ncube et al., 2008). Earlier studies have indicated the possibility of using botanical extracts for reducing white muscardine disease and effective in inhibiting the germination of spores. An aqueous extracts of *Terminalia chebula* fruit exhibit antifungal activity against a number of dermatophytes and yeast (Dutta et al., 1998). The broad spectrum of antibacterial activity was reported for *Azadirachta indica* (Singh et al., 2008). Bark of *P.hysterophorus* has been attributed to having cardio protective properties (Dwivedi, 2007). *Pongamia pinnata* seed extract are effective against tissue infection and skin diseases (Singh et al., 2009). Plant *Parthenium hysterophorus* is commonly found in India and used in folk medicine against skin infections. (Rahman et al., 1989, Teixeira et al., 1997). So in the present study the efficiency of aqueous extract of. *Azadirachta indica, parthenium hysterophorus, Ocimum sanctum and Pongamia Pinnata* is tested against larval characters, effective rate of rearing (ERR) and mortality in silkworm race $PMxNB_4D_2$ infected with *B. Bassiana.*

List of Plants

Table: 1.

Botanical Name	Local Name	Family	Part Used	Traditional Use
Azadirachta indica	Neem	Meliaceae	Seed	Immuno stimulant activity, anti fungal, anti ulcer, anti fertility, anti malarial, anti bacterial, anti viral, anti cancer
parthenium	Vayyari	Asteraceae	Leaves	Fever, diarrhea, neurologic

hysterophorus	Bhama			disorders, urinary tract infections, dysentery, malaria and as emmenagogue, inflammation, eczema, skin rashes, herpes, rheumatic pain, cold, heart trouble and gynecological ailments.
Pongamia Pinnata	Kanuga	Fabaceae	Seed	Heal scar tissue tumors, treat high blood pressure, and treat anemia powder reduces fever and helps in treating bronchitis
Osmium sanctum	Tulasi	Lameaceae	Leaves	Analgesic activity, anti-ulcer activity, anti arthritic activity, immune modulatory activity, anti asthmatic activity, anti fertility activity, anticancer activity, anti diabetic activity, anti hyper lipidemic activity, anti-inflammatory activity, antioxidant activity, anti stress activity.

MATERIALS AND METHODS

Selection of medicinal plants to study

In the present work few selected medicinal plants were screened for potential antifungal activity. These plants are listed in Table 1.

Identification and preservation of plant material

Fresh plant parts were collected from adjacent area of botany department, Acharya Nagarjuna University, Guntur Dist and A.P, shown in Plate 1. The taxonomic identification of the plants was made with available literature (Yadav and Sardesai 2002).

Preparation of plant extract

The collected plant material was washed with distilled water and shade dried at room temperature. The materials were grinded to fine powder with the help of mixer grinder. Then these powdered materials were used for preparation of aqueous extracts by using 50 g powder in 100 ml double distilled water for 72 h. The mixture was stirred every 24 h using a sterile glass rod. At the end of extraction each extract was concentrated in vacuum at 30ºC and stored at 4ºC until further use.

Preparation of pathogenic suspension

The pure fungal spores grown on the broth were harvested on the tenth day of inoculation with sterile distilled water using a sterile glass rod and the suspension was filtered through double layered muslin cloth. The original conidial stock suspension was serially diluted in sterile distilled water of at least 1x109spores/ml.

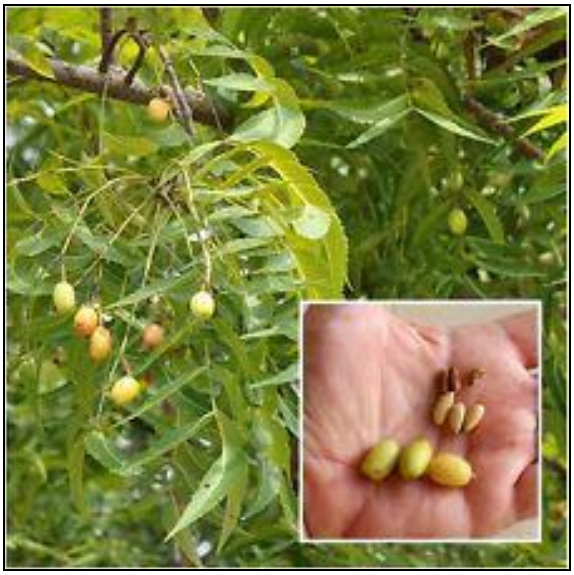
Artificial induction of fungal spores and plant extract to *Bombyx mori*

The *B. bassiana* infection was given to freshly moulted Vth instar larvae after two h starvation. The 60 larvae were taken in each tray and sprayed with the LD50 concentration of fungal spores. After 6 h of treatment the larvae were segregated to appropriate group and deeped in 5000 ppm concentration of different plant extract. One group was kept as inoculated control in which larvae not deeped in any plant extract and another group was kept as normal control in which larvae reared on fresh mulberry leaves without any spraying. The treatment of plant extracts are given for three days at morning time.

RESULTS AND DISCUSSION

The botanical extracts have exhibited significant results on larval character, effective rate of rearing and extent of mortality. The larval character showed significant results in increasing larval weight from all the botanical extract treated groups as per with normal control than the inoculated control (Table 2). Patil et al. (1997) reported that extrafoliation leaf water extract of *Parthenium* sp. has increased the larval weight, ERR of *B. mori*. Murugan et al. (1994) observed that extra foliation of aqueous extract of leaves of *pongamia pinnata* and *Ocimum sanctam* increased the total body weight of *B. mori*. These reports are in agreement with the present finding where the aqueous plant extract showed increase in larval weight gram per 10 larvae. In the present study all the plant extracts shows significant ERR and among these four.

Pongamia pinnata



Azadirachta indica

Ocimum sanctum

Parthenium hysterophorus

Plate 1. Plant parts collected from Acharya Nagarjuna University.

Plant extract *A. indica* recorded maximum ERR that is 71.66% and minimum was recorded for *O. sanctum* that is 60.66 % (Tables 3 to 4). Phytochemical compounds of these plants suppressed the spore formation of white muscardine. These results are in accordance or same line with the extrafoliation of *Psoralea coryleifolia* and *T. terrestris* at 800 ppm to mulberry leaves once during 3rd instar suppressed the grasserie disease by 80% (Sivaprakasam and Rabindra, 1996). Raghavaiah et al.

Table 2. Effect of plant extracts on larval weight of *Bombyx mori* L.

Group	Days						
	1	2	3	4	5	6	7
Normal control	8.62	14.92	26.59	31.39	37.11	41.09	43.79
Inoculated control	8.64	10.82	21.53	28.22	32.05	37.56	39.24
Azadirachta indica	8.60	13.08	22.40	29.20	33.71	41.09	42.22
Parthenium hysterophorus	8.65	13.76	22.26	29.46	34.59	37.29	38.82
Pongamia pinnata	8.68	13.06	22.28	29.57	35.76	38.76	40.55
Ocimum sanctum	7.62	17.44	20.19	31.51	34.89	40.12	40.30

Table 3: Effect of plant extracts on mortality and mortality in percentage of *Bombyx mori* L.

Groups	Mortality	Mortality (%)
Normal control	5	8.33
Inoculated control	32	54.44
Azadirachta indica	17	28.33
Parthenium hysterophorus	21	35
Pongamia pinnata	24	40
Ocimum sanctum	22	36.66

(1987) concluded that aqueous extract of *Allium sativum* (50%) was most effective in inhibiting the germination of spores of *B. bassiana.* All these results confirm the aqueous plant extract showed the disease reducing properties. This may be due to the presence of chemical constituent like, Alkaloids, Flavonoids, Glycosides, Steroids, Tannins, Eugenol, Farnesol, Geraniol, Rutin, Stigmasterol, Limonine, linalool, Hexacantanol, Jambosine, Arjunine, Lactone and Arjunetin, in

Groups	Mortality	Mortality (%)
Normal control	54	90.66
Inoculated control	22	41.33
Azadirachta indica	41	70.68
Parthenium hysterophorus	35	63.77
Pongamia pinnata	32	59.55
Ocimum sanctum	38	63.35

Azadirachta indica, parthenium hysterophorus, Ocimum sanctum and *Pongamia Pinnata* which helped in preventing the germination of spores of *B. bassiana* which indirectly helped in ERR (Singh et al., 2009; Patel and Kumar 2008; Ahmad and Beg, 2001). Further these results are agreement with Barnabas and Nagrajan (1958) they reported that the flavonoid rich extracts prepared from *Eucalyptus teriticornis* exhibited antifungal and antibacterial activity against several test organisms including *Aspergillus flavus* and *Candida albicam*. Hence in the present study some of the plant extracts having antifungal properties were identified which could be an effective tool in improving the general health of the silkworm and they might be used in reducing mortality and improving their economic parameters in muscardine infected larvae under *in vivo* condition by decreasing the disease incidence.

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